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BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the nematodes listed here.[5] Exposure to aerosolized sensitizing antigens of ascarids should be avoided. Primary containment (e.g., BSC) is recommended for work that may result in aerosolization of sensitization from occurring.

Special Issues

Treatment Highly effective medical treatment for most nematode infections exists.[4]

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 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-D: Rickettsial Agents

Coxiella burnetii

Coxiella burnetii is the etiologic agent of Q fever. *C. burnetii* is a bacterial obligate intracellular pathogen that undergoes its developmental cycle within an acidic vacuolar compartment exhibiting many characteristics of a phagolysosome. The developmental cycle consists of a large (approximately 1 µm in length) cell variant that is believed to be the more metabolically active, replicative cell type and a smaller, more structurally stable cell variant that is highly infectious and quite resistant to drying and environmental conditions.[1-4] The organism undergoes a virulent (Phase I) to avirulent (Phase II) transition upon serial laboratory passage in eggs or tissue culture.

The infectious dose of virulent Phase I organisms in laboratory animals has been calculated to be as small as a single organism.[5] The estimated human infectious dose for Q fever by inhalation is approximately 10 organisms.[6] Typically, the disease manifests with flu-like symptoms including fever, headache, and myalgia but can also cause pneumonia and hepatomegaly. Infections range from sub-clinical to severe although primary infections respond readily to antibiotic treatment. Although rare, *C. burnetii* is known to cause chronic infections such as endocarditis or granulomatous hepatitis.[7]

Occupational Infections

Q fever is the second most commonly reported LAI in Pike's compilation. Outbreaks involving 15 or more persons were recorded in several institutions.[8,9] Infectious aerosols are the most likely route of laboratory-acquired infections. Experimentally infected animals also may serve as potential sources of infection or laboratory and animal care personnel. Exposure to naturally infected, often fasymptomatic sheep and their birth products is a documented hazard to personnel.[10,11]

Natural Modes of Infection

Q fever (Q for query) occurs worldwide. Broad ranges of domestic and wild mammals are natural hosts for Q fever and sources of human infection. Parturient animals and their birth products are common sources of infection. The placenta of infected sheep may contain as many as 109 organisms per gram of tissue[12] and milk may contain 105 organisms per gram. The resistance of the organism to drying and its low infectious dose can lead to dispersal from contaminated sites.

Laboratory Safety and Containment Recommendations

The necessity of using embryonated eggs or cell culture techniques for the propagation of *C. burnetii* leads to extensive purification procedures. Exposure to infectious aerosols and parenteral inoculation cause most infections in laboratory and animal care personnel.[8,9] The agent may be present in infected arthropods

and in the blood, urine, feces, milk, and tissues of infected animals or human hosts. Exposure to naturally infected, often asymptomatic, sheep and their birth products is a documented hazard to personnel.[10,11] Recommended precautions for facilities using sheep as experimental animals are described elsewhere.[10,13]

BSL-2 practices and facilities are recommended for nonpropagative laboratory procedures, including serological examinations and staining of impression smears. BSL-3 practices and facilities are recommended for activities involving the inoculation, incubation, and harvesting of embryonated eggs or cell cultures, the necropsy of infected animals and the manipulation of infected tissues. Experimentally infected animals should be maintained under ABSL-3 because infected rodents may shed the organisms in urine or feces.[8] A specific plaque-purified clonal isolate of an avirulent (Phase II) strain (Nine Mile) may be safely handled under BSL-2 conditions.[14]

Special Issues

Vaccines An investigational Phase I, Q fever vaccine (IND) is available on a limited basis from the Special Immunizations Program (301-619-4653) of the USAMRIID, Fort Detrick, Maryland, for at-risk personnel under a cooperative agreement with the individual's requesting institution. The use of this vaccine should be restricted to those who are at high risk of exposure and who have no demonstrated sensitivity to Q fever antigen. The vaccine can be reactogenic in those with prior immunity, thus requires skin testing before administration. The vaccine is only administered at USAMRIID and requires enrollment in their Q fever IND Immunization Program. For at-risk laboratory workers to participate in this program, fees are applicable. Individuals with valvular heart disease should not work with *C. burnetii.* (See Section VII.)

Select Agent *C. burnetii* is a select agent 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 this agent may require CDC and/or USDA 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.

Rickettsia prowazekii; Rickettsia typhi (R. mooseri); Orientia (Rickettsia) tsutsugamushi and Spotted Fever Group agents of human disease; Rickettsia rickettsii, Rickettsia conorii, Rickettsia akari, Rickettsia australis, Rickettsia siberica, and Rickettsia japonicum

Rickettsia prowazekii, Rickettsia typhi (R. mooseri), Orientia (Rickettsia) tsutsugamushi and the Spotted Fever Group agents of human disease (*Rickettsia rickettsii, Rickettsia conorii, Rickettsia akari, Rickettsia australis, Rickettsia siberica,* and

Rickettsia japonicum) are the etiologic agents of epidemic typhus, endemic (murine) typhus), scrub typhus, Rocky Mountain spotted fever, Mediterranean spotted fever, rickettsialpox, Queensland tick typhus, and North Asian spotted fever, respectively.

Rickettsia spp. are bacterial obligate intracellular pathogens that are transmitted by arthropod vectors and replicate within the cytoplasm of eukaryotic host cells. Two groups are recognized within the genus, the typhus group and the spotted fever group. The more distantly related scrub typhus group is now considered a distinct genus, *Orientia.* Rickettsiae are primarily associated with arthropod vectors in which they may exist as endosymbionts that infect mammals, including humans, through the bite of infected ticks, lice, or fleas.[15]

Occupational Infections

Pike reported 57 cases of laboratory-associated typhus (type not specified), 56 cases of epidemic typhus with three deaths, and 68 cases of murine typhus.[8] Three cases of murine typhus have been reported from a research facility.[16] Two were associated with handling of infectious materials on the open bench; the third case resulted from an accidental parenteral inoculation. These three cases represented an attack rate of 20% in personnel working with infectious materials. Rocky Mountain spotted fever is a documented hazard to laboratory personnel. Pike reported 63 laboratory-associated cases, 11 of which were fatal.[8] Oster reported nine cases occurring over a six-year period in one laboratory. All were believed to have been acquired because of exposure to infectious aerosols.[17]

Natural Modes of Infection

The epidemiology of rickettsial infections reflects the prevalence of rickettsiae in the vector population and the interactions of arthropod vectors with humans. Epidemic typhus is unusual among rickettsiae in that humans are considered the primary host. Transmission is by the human body louse; thus, outbreaks are now associated with breakdowns of social conditions. Endemic typhus is maintained in rodents and transmitted to humans by fleas. The various spotted fever group rickettsiae are limited geographically, probably by the distribution of the arthropod vector, although specific spotted fever group rickettsiae are found on all continents.[15]

Laboratory Safety and Containment Recommendations

The necessity of using embryonated eggs or cell culture techniques for the propagation of *Rickettsia* spp. incorporates extensive purification procedures. Accidental parenteral inoculation and exposure to infectious aerosols are the most likely sources of LAI.[18] Aerosol transmission of *R. rickettsii* has been experimentally documented in nonhuman primates.[19] Five cases of rickettsialpox recorded by Pike were associated with exposure to bites of infected mites.[8] Naturally and experimentally infected mammals, their ectoparasites, and their infected tissues are potential sources of human infection. The organisms are relatively unstable under ambient environmental conditions.

BSL-2 practices, containment equipment, and facilities are recommended for nonpropagative laboratory procedures, including serological and fluorescent antibody procedures, and for the staining of impression smears. BSL-3 practices, containment equipment, and facilities are recommended for all other manipulations of known or potentially infectious materials, including necropsy of experimentally infected animals and trituration of their tissues, and inoculation, incubation, and harvesting of embryonated eggs or cell cultures. ABSL-2 practices, containment equipment, and facilities are recommended for the holding of experimentally infected mammals other than arthropods. BSL-3 practices, containment equipment, and facilities are recommended for animal studies with arthropods naturally or experimentally infected with rickettsial agents of human disease. (See Appendix E.)

Several species, including *R. montana, R. rhipicephali, R. belli*, and *R. canada,* are not known to cause human disease and may be handled under BSL-2 conditions. New species are being described frequently and should be evaluated for appropriate containment on a case-by-case basis. Because of the proven value of antibiotic therapy in the early stages of ricketsial infection, it is essential that laboratories have an effective system for reporting febrile illnesses in laboratory personnel, medical evaluation of potential cases and, when indicated, institution of appropriate antibiotic therapy.

Special Issues

Medical Response Under natural circumstances, the severity of disease caused by rickettsial agents varies considerably. In the laboratory, very large inocula are possible, which might produce unusual and perhaps very serious responses. Surveillance of personnel for laboratory-associated infections with rickettsial agents can dramatically reduce the risk of serious consequences of disease. Experience indicates that infections adequately treated with specific anti-rickettsial chemotherapy on the first day of disease do not generally present serious problems. However, delay in instituting appropriate chemotherapy may result in debilitating or severe acute disease ranging from increased periods of convalescence in typhus and scrub typhus to death in *R. rickettsii* infections. The key to reducing the severity of disease from laboratory-associated infections is a reliable medical response which includes: 1) round-the-clock availability of an experienced medical officer; 2) indoctrination of all personnel on the potential hazards of working with rickettsial agents and advantages of early therapy; 3) a reporting system for all recognized overt exposures and accidents; 4) the reporting of all febrile illnesses, especially those associated with headache, malaise, and prostration when no other certain cause exists; and 5) an open and non-punitive atmosphere that encourages reporting of any febrile illness.

Select Agent *R. prowazekii* and *R. rickettsii* 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 this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

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Section VIII-E: Viral Agents

Hantaviruses

Hantaviruses are negative sense RNA viruses belonging to the genus *Hantavirus* within the family *Bunyaviridae.* The natural hosts of hantaviruses are rodent species and they occur worldwide. Hantavirus pulmonary syndrome (HPS) is a severe disease caused by hantaviruses such as Sin Nombre virus or Andes virus whose hosts are rodents in the subfamily *Sigmodontinae.* This subfamily only occurs in the New World, so HPS is not seen outside North and South America. Hantaviruses in Europe and Asia frequently cause kidney disease, called nephropathica epidemica in Europe, and hemorrhagic fever with renal syndrome (HFRS) in Asia.

Occupational Infections

Documented laboratory-acquired infections have occurred in individuals working with hantaviruses.[1-4] Extreme caution must be used in performing any laboratory operation that may create aerosols (centrifugation, vortex-mixing, etc.). Operations involving rats, voles, and other laboratory rodents, should be conducted with special caution because of the extreme hazard of aerosol infection, especially from infected rodent urine.

Natural Modes of Infection

HPS is a severe, often fatal disease that is caused by Sin Nombre and Andes or related viruses.[5,6] Most cases of human illness have resulted from exposures to naturally infected wild rodents or to their excreta. Person-to-person transmission does not occur, with the exception of a few rare instances documented for Andes virus.[7] Arthropod vectors are not known to transmit hantaviruses.

Laboratory Safety and Containment Recommendations

Laboratory transmission of hantaviruses from rodents to humans via the aerosol route is well documented.[4-7] Exposures to rodent excreta, especially aerosolized infectious urine, fresh necropsy material, and animal bedding are presumed to be associated with risk. Other potential routes of laboratory infection include ingestion, contact of infectious materials with mucous membranes or broken skin and, in particular, animal bites. Viral RNA has been detected in necropsy specimens and in patient blood and plasma obtained early in the course of HPS;[8,9] however, the infectivity of blood or tissues is unknown.

BSL-2 practices, containment equipment, and facilities are recommended for laboratory handling of sera from persons potentially infected with hantaviruses. The use of a certified BSC is recommended for all handling of human body fluids when potential exists for splatter or aerosol.

Potentially infected tissue samples should be handled in BSL-2 facilities following BSL-3 practices and procedures. Cell-culture virus propagation and purification should be carried out in a BSL-3 facility using BSL-3 practices, containment equipment and procedures.

Experimentally infected rodent species known not to excrete the virus can be housed in ABSL-2 facilities using ABSL-2 practices and procedures. Primary physical containment devices including BSCs should be used whenever procedures with potential for generating aerosols are conducted. Serum or tissue samples from potentially infected rodents should be handled at BSL-2 using BSL-3 practices, containment equipment and procedures. All work involving inoculation of virus-containing samples into rodent species permissive for chronic infection should be conducted at ABSL-4.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Hendra Virus (formerly known as Equine Morbillivirus) and Nipah Virus

Hendra virus and *Nipah* virus are members of a newly recognized genus called *Henipavirus,* within the family *Paramyxoviridae.* Outbreaks of a previously unrecognized paramyxovirus, at first called equine morbillivirus, later named Hendra virus, occurred in horses in Australia in 1994 and 1995. During 1998-1999, an outbreak of illness caused by a similar but distinct virus, now known as Nipah virus, occurred in Malaysia and Singapore. Human illness, characterized by fever, severe headache, myalgia and signs of encephalitis occurred in individuals in close contact with pigs (i.e., pig farmers and abattoir workers).[10-14] A few patients developed a respiratory disease. Approximately 40% of patients with encephalitis died. Recently, cases of Nipah virus infection were described in Bangladesh, apparently the result of close contact with infected fruit bats without an intermediate (e.g., pig) host.

Occupational Infections

No laboratory-acquired infections are known to have occurred because of Hendra or Nipah virus exposure; however, three people in close contact with ill horses developed encephalitis or respiratory disease and two died.[15-20]

Natural Modes of Infection

The natural reservoir hosts for the Hendra and Nipah viruses appear to be fruit bats of the genus *Pteropus*.[21-23] Studies suggest that a locally occurring member

of the genus, *Pteropus giganteus*, is the reservoir for the virus in Bangladesh.[24] Individuals who had regular contact with bats had no evidence of infection (antibody) in one study in Australia.[25]

Laboratory Safety and Containment Recommendations

The exact mode of transmission of these viruses has not been established. Most clinical cases to date have been associated with close contact with horses, their blood or body fluids (Australia) or pigs (Malaysia/Singapore) but presumed direct transmission from *Pteropus* bats has been recorded in Bangladesh. Hendra and Nipah viruses have been isolated from tissues of infected animals. In the outbreaks in Malaysia and Singapore, viral antigen was found in central nervous system, kidney and lung tissues of fatal human cases[26] and virus was present in secretions of patients, albeit at low levels.[27] Active surveillance for infection of healthcare workers in Malaysia has not detected evidence of occupationally acquired infections in this setting.[28]

Because of the unknown risks to laboratory workers and the potential impact on indigenous livestock should the virus escape a diagnostic or research laboratory, health officials and laboratory managers should evaluate the need to work with the virus and the containment capability of the facility before undertaking any work with Hendra, Nipah or suspected related viruses. BSL-4 is required for all work with these viruses. Once a diagnosis of Nipah or Hendra virus is suspected, all diagnostic specimens also must be handled at BSL-4. ABSL-4 is required for any work with infected animals.

Special Issues

Select Agent Hendra and Nipah virus 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 this agent may require CDC and/or USDA 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.

Hepatitis A Virus, Hepatitis E Virus

Hepatitis A virus is a positive single-stranded RNA virus, the type species of the Hepatovirus genus in the family Picornaviridae. Hepatitis E virus is a positive single-stranded RNA virus, the type species of the genus Hepevirus, a floating genus not assigned to any family.

Occupational Infections

Laboratory-associated infections with hepatitis A or E viruses do not appear to be an important occupational risk among laboratory personnel. However, hepatitis A is a documented hazard in animal handlers and others working with naturally or experimentally infected chimpanzees and other nonhuman primates.[29] Workers handling other recently captured, susceptible primates (owl monkeys, marmosets) also may be at risk for hepatitis A infection. Hepatitis E virus appears to be less of a risk to personnel than hepatitis A virus, except during pregnancy, when infection can result in severe or fatal disease.

Natural Modes of Infection

Most infections with hepatitis A are foodborne and occasionally water-borne. The virus is present in feces during the prodromal phase of the disease and usually disappears once jaundice occurs. Hepatitis E virus causes acute enterically-transmitted cases of hepatitis, mostly waterborne. In Asia, epidemics involving thousands of cases have occurred.

Laboratory Safety and Containment Recommendations

The agents may be present in feces and blood of infected humans and nonhuman primates. Feces, stool suspensions, and other contaminated materials are the primary hazards to laboratory personnel. Care should be taken to avoid puncture wounds when handling contaminated blood from humans or nonhuman primates. There is no evidence that aerosol exposure results in infection.

BSL-2 practices, containment equipment, and facilities are recommended for the manipulation of hepatitis A and E virus, infected feces, blood or other tissues. ABSL-2 practices and facilities are recommended for activities using naturally or experimentally-infected nonhuman primates or other animal models that may shed the virus.

Special Issues

Vaccines A licensed inactivated vaccine against hepatitis A is available. Vaccines against hepatitis E are not currently available.

Transfer of Agent Importation of this agent may require CDC and/or USDA 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.

Hepatitis B Virus, Hepatitis C Virus (formerly known as nonA nonB Virus), Hepatitis D Virus

Hepatitis B virus (HBV) is the type species of the *Orthohepadnavirus* genus in the family *Hepadnaviridae*. Hepatitis C virus (HCV) is the type species of the *Hepacivirus* genus in the family *Flaviviridae.* Hepatitis D virus (HDV) is the only member of the genus *Deltavirus.*

These viruses are naturally acquired from a carrier during blood transfusion, vaccination, tattooing, or body piercing with inadequately sterilized instruments. Non-parenteral routes, such as domestic contact and unprotected (heterosexual and homosexual) intercourse, are also major modes of transmission.

Individuals who are infected with the HBV are at risk of infection with HDV, a defective RNA virus that requires the presence of HBV virus for replication. Infection with HDV usually exacerbates the symptoms caused by HBV infection.

Occupational Infections

Hepatitis B has been one of the most frequently occurring laboratory-associated infections, and laboratory workers are recognized as a high-risk group for acquiring such infections.[30]

Hepatitis C virus infection can occur in the laboratory situation as well.[31] The prevalence of antibody to hepatitis C (anti-HCV) is slightly higher in medical care workers than in the general population. Epidemiologic evidence indicates that HCV is spread predominantly by the parenteral route.[32]

Laboratory Safety and Containment Recommendations

HBV may be present in blood and blood products of human origin, in urine, semen, CSF and saliva. Parenteral inoculation, droplet exposure of mucous membranes, and contact exposure of broken skin are the primary laboratory hazards.[33] The virus may be stable in dried blood or blood components for several days. Attenuated or avirulent strains have not been identified.

HCV has been detected primarily in blood and serum, less frequently in saliva and rarely or not at all in urine or semen. It appears to be relatively unstable to storage at room temperature and repeated freezing and thawing.

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids and tissues. Additional primary containment and personnel precautions, such as those described for BSL-3, may be indicated for activities with potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials. ABSL-2 practices, containment equipment and facilities are recommended for activities utilizing naturally or experimentally infected chimpanzees or other NHP. Gloves should be worn when working with

infected animals and when there is the likelihood of skin contact with infectious materials. In addition to these recommended precautions, persons working with HBV, HCV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogen Standard.[34] Questions related to interpretation of this Standard should be directed to federal, regional or state OSHA offices.

Special Issues

Vaccines Licensed recombinant vaccines against hepatitis B are available and are highly recommended for and offered to laboratory personnel.[35] Vaccines against hepatitis C and D are not yet available for use in humans, but vaccination against HBV will also prevent HDV infection.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Herpesvirus Simiae (Cerocopithecine Herpesvirus I, Herpes B Virus)

B virus is a member of the *alphaherpesvirus* genus (simplexvirus) in the family *Herpesviridae.* It occurs naturally in macaque monkeys, of which there are nine distinct species. Macaques may have primary, recurrent, or latent infections often with no apparent symptoms or lesions. B virus is the only member of the family of simplex herpesviruses that can cause zoonotic infections. Human infections have been identified in at least 50 instances, with approximately 80% mortality when untreated. There remains an approximate 20% mortality in the absence of timely treatment with antiviral agents.[36] There have been no reported cases where prompt first aid with wound or exposure site cleansing was performed, and no cases where cleaning and post exposure prophylaxis were done. Cases prior to 1970 were not treated with antiviral agents because none were available. Morbidity and mortality associated with zoonotic infection results from invasion of the central nervous system, resulting in ascending paralysis ultimately with loss of ability to sustain respiration in the absence of mechanical ventilation. From 1987-2004, five additional fatal infections bring the number of lethal infections to 29 since the discovery of B virus in 1933.

Occupational Infections

B virus is a hazard in facilities where macaque monkeys are present. Mucosal secretions (saliva, genital secretions, and conjunctival secretions) are the primary body fluids associated with risk of B virus transmission. However, it is possible for other materials to become contaminated. For instance, a research assistant at the Yerkes Primate Center who died following mucosal splash without injury in 1997 was splashed with something in the eye while transporting a caged macaque. In part on this basis, the eye splash was considered low risk. However,

feces, urine or other fluids may be contaminated with virus shed from mucosal fluids. Zoonoses have been reported following virus transmission through a bite, scratch, or splash accident. Cases of B virus have also been reported after exposure to monkey cell cultures and to central nervous system tissue. There is often no apparent evidence of B virus infection in the animals or their cells and tissues, making it imperative that all suspect exposures be treated according to recommended standards.[36] The risks associated with this hazard are, however, readily reduced by practicing barrier precautions and by rapid and thorough cleansing immediately following a possible site contamination. Precautions should be followed when work requires the use of any macaque species, even antibody negative animals. In most documented cases of B virus zoonosis, virus was not recovered from potential sources except in four cases, making speculations that some macaque species may be safer than others unfounded. The loss of five lives in the past two decades underscores that B virus infections have a low probability of occurrence, but when they do occur it is with high consequences.

Specific, regular training in risk assessments for B virus hazards including understanding the modes of exposure and transmission should be provided to individuals encountering B virus hazards. This training should include proper use of personal protective equipment, which is essential to prevention. Immediate and thorough cleansing following bites, scratches, splashes, or contact with potential fomites in high-risk areas appears to be helpful in prevention of B virus infections.[37] First aid and emergency medical assistance procedures are most effective when institutions set the standard to be practiced by all individuals encountering B virus hazards.

Natural Modes of Infection

B virus occurs as a natural infection of Asiatic macaque monkeys, and some 10% of newly caught rhesus monkeys have antibodies against the virus, which is frequently present in kidney cell cultures of this animal.

Reservoir species include *Macaca mulatta*, *M. fascicularis, M.fusata, M. arctoides, M. cyclopsis* and *M. radiata.* In these species the virus causes vesicular lesions on the tongue and lips, and sometimes of the skin. B virus is not present in blood or serum in infected macaques. Transmission of B virus appears to increase when macaques reach sexual maturity.

Laboratory Safety and Containment Recommendations

The National Academies Press has recently published ILAR's guidelines for working with nonhuman primates.[38] Additional resources are provided in the references following this agent summary statement. Asymptomatic B virus shedding accounts for most transmission among monkeys and human workers, but those working in the laboratory with potentially infected cells or tissues from macaques are also at risk. Exposure of mucous membranes or through skin

breaks provides this agent access to a new host, whether the virus is being shed from a macaque or human, or present in or on contaminated cells, tissues, or surfaces.[36] B virus is not generally found in serum or blood, but these products obtained through venipuncture should be handled carefully because contamination of needles via skin can occur. When working with macaques directly, virus can be transmitted through bites, scratches, or splashes only when the animal is shedding virus from mucosal sites. Fomites, or contaminated surfaces (e.g., cages, surgical equipment, tables), should always be considered sources of B virus unless verified as decontaminated or sterilized. Zoonotically infected humans should be cautioned about autoinoculation of other susceptible sites when shedding virus during acute infection.

BSL-2 practices and facilities are suitable for all activities involving the use or manipulation of tissues, cells, blood, or serum from macaques with appropriate personal protective equipment. BSL-3 practices are recommended for handling materials from which B virus is being cultured using appropriate personal protective equipment, and BSL-4 facilities are recommended for propagation of virus obtained from diagnostic samples or stocks. Experimental infections of macaques as well as small animal models with B virus are recommended to be restricted to BSL-4 containment.

All macaques regardless of their origin should be considered potentially infected. Animals with no detectable antibody are not necessarily B virus-free. Macaques should be handled with strict barrier precaution protocols and injuries should be tended immediately according to the recommendations of the B Virus Working Group led by NIH and CDC.[36]

Barrier precautions and appropriate first aid are the keys to prevention of severe morbidity and mortality often associated with B virus zoonoses. These prevention tools were not implemented in each of the five B virus fatalities during the past two decades. Guidelines are available for safely working with macaques and should be consulted.[36,39] The correct use of gloves, masks, and protective coats, gowns, aprons, or overalls is recommended for all personnel while working with non-human primates, especially macaques and other Old World species, including for all persons entering animal rooms where non-human primates are housed. To minimize the potential for mucous membrane exposure, some form of barrier is required to prevent droplet splashes to eyes, mouth, and nasal passages. Types and use of personal protective equipment (e.g., goggles or glasses with solid side shields and masks, or wrap-around face shields) should be determined with reference to the institutional risk assessment. Specifications of protective equipment must be balanced with the work to be performed so that the barriers selected do not increase work place risk by obscuring vision and contributing to increased risk of bites, needle sticks, scratches, or splashes.

Special Issues

Post-exposure prophylaxis with oral acyclovir or valacyclovir should be considered for significant exposures to B virus. Therapy with intravenous acyclovir and/or ganciclovir in documented B virus infections is also important in reduction of morbidity following B virus zoonotic infection.[36] In selected cases, IND permission has been granted for therapy with experimental antiviral drugs. Because of the seriousness of B virus infection, experienced medical and laboratory personnel should be consulted to develop individual case management. Barrier precautions should be observed with confirmed cases. B virus infection, as with all alphaherpesviruses, is lifelong in macaques.[40] There are no effective vaccines available.

Select Agent B virus is a select agent 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 this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Human Herpes Virus

The herpesviruses are ubiquitous human pathogens and are commonly present in a variety of clinical materials submitted for virus isolation. Thus far, nine herpesviruses have been isolated from humans: herpes simplex virus-1(HSV-1), HSV-2, human cytomegalovirus (HCMV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), and human herpesviruses (HHV) 6A, 6B, 7, and 8.[41]

HSV infection is characterized by a localized primary lesion. Primary infection with HSV-1 may be mild and unapparent occurring in early childhood. In approximately 10% of infections, overt illness marked by fever and malaise occurs. HSV-1 is a common cause of meningoencephalitis. Genital infections, usually caused by HSV-2, generally occur in adults and are sexually transmissible. Neonatal infections are most frequently caused by HSV-2 but HSV-1 infections are also common. In the neonate, disseminated disease and encephalitis are often fatal. EBV is the cause of infectious mononucleosis. It is also associated with the pathogenesis of several lymphomas and nasopharyngeal cancer.[42] EBV is serologically distinct from the other herpesviruses; it infects and transforms B-lymphocytes. HCMV infection is common and often undiagnosed presenting as a nonspecific febrile illness. HCMV causes up to 10% of all cases of mononucleosis in young adults. The most severe form of the disease is seen in infants infected *in utero*. Children surviving infection may evidence mental retardation, microencephaly, motor disabilities and chronic liver disease.[42] HCMV is one of the most common congenital diseases.

VZV is the causative agent of chickenpox and herpes zoster. Chickenpox usually occurs in childhood and zoster occurs more commonly in adults. HHV-6 is the causative agent of exanthema subitum (roseola), a common childhood exanthem.[43] Nonspecific febrile illness and febrile seizures are also clinical manifestations of disease. HHV-6 may reactivate in immunocompetent individuals during pregnancy or during critical illness. Two distinct variants, HHV-6A and HHV-6B, exist, the latter causing roseola. HHV-7 is a constitutive inhabitant of adult human saliva.[44] Clinical manifestations are less well understood but the virus has also been associated with roseola. HHV-8, also known as Kaposi's sarcoma-associated virus, was first identified by Chang and co-workers in 1994.[42] HHV-8 is believed to be the causative agent of Kaposi's sarcoma and has been associated with primary effusion lymphoma.[45] The natural history of HHV-8 has not been completely elucidated. High risk groups for HHV-8 include HIV-infected men who have sex with men and individuals from areas of high endemicity, such as Africa or the Mediterranean.[45] The prevalence of HHV-8 is also higher among intravenous drug users than in the general population.[45] At least one report has provided evidence that in African children, HHV-8 infection may be transmitted from mother to child.[46] While few of the human herpesviruses have been demonstrated to cause laboratory-acquired infections, they are both primary and opportunistic pathogens, especially in immunocompromised hosts. Herpesvirus simiae (B-virus, Monkey B virus) is discussed separately in another agent summary statement in this section.

Occupational Infections

Few of the human herpesviruses have been documented as sources of laboratory acquired infections.

In a limited study, Gartner and co-workers have investigated the HHV-8 immunoglobulin G (IgG) seroprevalence rates for healthcare workers caring for patients with a high risk for HHV-8 infection in a non-endemic area. Healthcare workers in contact with risk group patients were infected more frequently than healthcare workers without contact with risk groups. Workers without contact with risk group patients were infected no more frequently than the control group.[53]

Although this diverse group of indigenous viral agents has not demonstrated a high potential hazard for laboratory-associated infection, frequent presence in clinical materials and common use in research warrant the application of appropriate laboratory containment and safe practices.

Natural Modes of Infection

Given the wide array of viruses included in this family, the natural modes of infection vary greatly, as does the pathogenesis of the various viruses. Some have wide host ranges, multiply effectively, and rapidly destroy the cells they infect (HSV-1, HSV-2). Others have restricted host ranges or long replicative

cycles (HHV-6).[41] Transmission of human herpesviruses in nature are, in general, associated with close, intimate contact with a person excreting the virus in their saliva, urine, or other bodily fluids.[47] VZV is transmitted person-to-person through direct contact, through aerosolized vesicular fluids and respiratory secretions, and indirectly transmitted by fomites. Latency is a trait common to most herpesviruses, although the site and duration vary greatly. For example, EBV will persist in an asymptomatic, latent form in the host immune system, primarily in EBV-specific cytotoxic T cells[42] while latent HSV has been detected only in sensory neurons.[48,49] HHV-8 has been transmitted through organ transplantation[50] and blood transfusion;[51] some evidence suggests non-sexual horizontal transmission.[52]

Laboratory Safety and Containment Recommendations

Clinical materials and isolates of herpesviruses may pose a risk of infection following ingestion, accidental parenteral inoculation, and droplet exposure of the mucous membranes of the eyes, nose, or mouth, or inhalation of concentrated aerosolized materials. HHV-8 may be present in human blood or blood products and tissues or saliva. Aerosol transmission cannot be excluded as a potential route of transmission. Clinical specimens containing the more virulent Herpesvirus simiae (B-virus) may be inadvertently submitted for diagnosis of suspected herpes simplex infection. HCMV may pose a special risk during pregnancy because of potential infection of the fetus. All human herpesviruses pose an increased risk to persons who are immunocompromised.

BSL-2 practices, containment equipment, and facilities are recommended for activities utilizing known or potentially infectious clinical materials or cultures of indigenous viral agents that are associated or identified as a primary pathogen of human disease. Although there is little evidence that infectious aerosols are a significant source of LAI, it is prudent to avoid the generation of aerosols during the handling of clinical materials or isolates, or during the necropsy of animals. Primary containment devices (e.g., BSC) should be utilized to prevent exposure of workers to infectious aerosols. Additional containment and procedures, such as those described for BSL-3, should be considered when producing, purifying, and concentrating human herpesviruses, based on risk assessment.

Containment recommendations for herpesvirus simiae (B-virus, Monkey B virus) are described in the preceding agent summary statement.

Special Issues

Vaccine A live, attenuated vaccine for varicella zoster is licensed and available in the United States. In the event of a laboratory exposure to a non-immune individual, varicella vaccine is likely to prevent or at least modify disease.[47]

Treatment Antiviral medications are available for treatment of several of the herpesviruses.

Transfer of Agent Importation of this agent may require CDC and/or USDA 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.

Influenza

Influenza is an acute viral disease of the respiratory tract. The most common clinical manifestations are fever, headache, malaise, sore throat and cough. GI tract manifestations (nausea, vomiting and diarrhea) are rare but may accompany the respiratory phase in children. The two most important features of influenza are the epidemic nature of illness and the mortality that arises from pulmonary complications of the disease.[54]

The influenza viruses are enveloped RNA viruses belonging to the Orthomyxoviridae. There are three serotypes of influenza viruses, A, B and C. Influenza A is further classified into subtypes by the surface glycoproteins that possess either hemagglutinin (H) or neuraminidase (N) activity. Emergence of completely new subtypes (antigenic shift) occurs at irregular intervals with Type A viruses. New subtypes are responsible for pandemics and can result from reassortment of human and avian influenza virus genes. Antigenic changes within a type or subtype (antigenic drift) of A and B viruses are ongoing processes that are responsible for frequent epidemics and regional outbreaks and make the annual reformulation of influenza vaccine necessary.

Influenza viral infections, with different antigenic subtypes, occur naturally in swine, horses, mink, seals and in many domestic and wild avian species. Interspecies transmission and reassortment of influenza A viruses have been reported to occur among humans and wild and domestic fowl. The human influenza viruses responsible for the 1918, 1957 and 1968 pandemics contained gene segments closely related to those of avian influenza viruses.[55] Swine influenza has also been isolated in human outbreaks.[56]

Control of influenza is a continuing human and veterinary public health concern.

Occupational Infections

LAI have not been routinely documented in the literature, but informal accounts and published reports indicate that such infections are known to have occurred, particularly when new strains showing antigenic shift or drift are introduced into a laboratory for diagnostic/research purposes.[56] Occupationally-acquired, nosocomial infections are documented.[57,58] Laboratory animal-associated infections have not been reported; however, there is possibility of human infection acquired from infected ferrets and vice versa.

Natural Modes of Infection

Airborne spread is the predominant mode of transmission especially in crowded, enclosed spaces. Transmission may also occur through direct contact since influenza viruses may persist for hours on surfaces particularly in the cold and under conditions of low humidity.[55] The incubation period is from one to three days. Recommendations for treatment and prophylaxis of influenza are available.[59]

Laboratory Safety and Containment Recommendations

The agent may be present in respiratory tissues or secretions of humans and most infected animals and birds. In addition, the agent may be present in the intestines and cloacae of many infected avian species. Influenza viruses may be disseminated in multiple organs in some infected animal species. The primary laboratory hazard is inhalation of virus from aerosols generated by infecting animals or by aspirating, dispensing, mixing, centrifuging or otherwise manipulating virus-infected samples. In addition, laboratory infection can result from direct inoculation of mucus membranes through virus-contaminated gloves following handling of tissues, feces or secretions from infected animals. Genetic manipulation has the potential for altering the host range, pathogenicity, and antigenic composition of influenza viruses. The potential for introducing influenza viruses with novel genetic composition into humans is unknown.

BSL-2 facilities, practices and procedures are recommended for diagnostic, research and production activities utilizing contemporary, circulating human influenza strains (e.g., H1/H3/B) and low pathogenicity avian influenza (LPAI) strains (e.g., H1-4, H6, H8-16), and equine and swine influenza viruses. ABSL-2 is appropriate for work with these viruses in animal models. All avian and swine influenza viruses require an APHIS permit. Based on economic ramifications and source of the virus, LPAI H5 and H7 and swine influenza viruses may have additional APHIS permit-driven containment requirements and personnel practices and/or restrictions.

Non-Contemporary Human Influenza (H2N2) Strains

Non-contemporary, wild-type human influenza (H2N2) strains should be handled with increased caution. Important considerations in working with these strains are the number of years since an antigenically related virus last circulated and the potential for presence of a susceptible population. BSL-3 and ABSL-3 practices, procedures and facilities are recommended with rigorous adherence to additional respiratory protection and clothing change protocols. Negative pressure, HEPA-filtered respirators or positive air-purifying respirators (PAPRs) are recommended for use. Cold-adapted, live attenuated H2N2 vaccine strains may continue to be worked with at BSL-2.

1918 Influenza Strain

Any research involving reverse genetics of the 1918 influenza strain should proceed with *extreme* caution. The risk to laboratory workers is unknown, but the pandemic potential is thought to be significant. Until further risk assessment data are available, the following practices and conditions are recommended for manipulation of reconstructed 1918 influenza viruses and laboratory animals infected with the viruses. These practices and procedures are considered minimum standards for work with the fully reconstructed virus.

- BSL-3 and ABSL-3 practices, procedures and facilities.
- Large laboratory animals such as NHP should be housed in primary barrier systems in ABSL-3 facilities.
- Rigorous adherence to additional respiratory protection and clothing change protocols.
- Use of negative pressure, HEPA-filtered respirators or PAPRs.
- Use of HEPA filtration for treatment of exhaust air.
- Amendment of personnel practices to include personal showers prior to exiting the laboratory.

Highly Pathogenic Avian Influenza (HPAI)

Manipulating HPAI viruses in biomedical research laboratories requires similar caution because some strains may pose increased risk to laboratory workers and have significant agricultural and economic implications. BSL-3 and ABSL-3 practices, procedures and facilities are recommended along with clothing change and personal showering protocols. Loose-housed animals infected with HPAI strains must be contained within BSL-3-Ag facilities. (See Appendix D.) Negative pressure, HEPA-filtered respirators or positive air-purifying respirators are recommended for HPAI viruses with potential to infect humans. The HPAI are agricultural select agents requiring registration of personnel and facilities with the lead agency for the institution (CDC or USDA-APHIS). An APHIS permit is also required. Additional containment requirements and personnel practices and/or restrictions may be added as conditions of the permit.

Other Influenza Recombinant or Reassortant Viruses

When considering the biocontainment level and attendant practices and procedures for work with other influenza recombinant or reassortant viruses, the local IBC should consider but not limit consideration to the following in the conduct of protocol-driven risk assessment.

- The gene constellation used.

- Clear evidence of reduced virus replication in the respiratory tract of appropriate animal models, compared with the level of replication of the wild-type parent virus from which it was derived.
- Evidence of clonal purity and phenotypic stability.
- The number of years since a virus that was antigenically related to the donor of the hemagglutinin and neuraminidase genes last circulated.

If adequate risk assessment data are not available, a more cautious approach utilizing elevated biocontainment levels and practices is warranted. There may be specific requirements regarding the setting of containment levels if your institution is subject to the *NIH Guidelines*.

Special Issues

Occupational Health Considerations Institutions performing work with HPAI and avian viruses that have infected humans; non-contemporary wild-type human influenza strains, including recombinants and reassortants; and viruses created by reverse genetics of the 1918 pandemic strain should develop and implement a specific medical surveillance and response plan. At the minimum these plans should: 1) require storage of baseline serum samples from individuals working with these influenza strains; 2) strongly recommend annual vaccination with the currently licensed influenza vaccine for such individuals; 3) provide employee counseling regarding disease symptoms including fever, conjunctivitis and respiratory symptoms; 4) establish a protocol for monitoring personnel for these symptoms; and 5) establish a clear medical protocol for responding to suspected laboratory-acquired infections. Antiviral drugs (e.g., oseltamivir, amantadine, rimantadine, zanamivir) should be available for treatment and prophylaxis, as necessary.[59] It is recommended that the sensitivities of the virus being studied to the antivirals be ascertained. All personnel should be enrolled in an appropriately constituted respiratory protection program.

Influenza viruses may require USDA and/or USPHS import permits depending on the host range and pathogenicity of the virus in question.

Select Agent Strains of HPAI and 1918 influenza virus 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 this agent may require CDC and/or USDA 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.

Lymphocytic Choriomeningitis Virus

Lymphocytic choriomeningitis (LCM) is a rodent-borne viral infectious disease that presents as aseptic meningitis, encephalitis, or meningoencephalitis. The causative agent is the LCM virus (LCMV) that was initially isolated in 1933. The virus is the protypical member of the family *Arenaviridae.*

Occupational Infections

LAI with LCM virus are well documented. Most infections occur when chronic viral infection exists in laboratory rodents, especially mice, hamsters and guinea pigs.[60-62] Nude and severe combined immune deficient (SCID) mice may pose a special risk of harboring silent chronic infections. Inadvertently infected cell cultures also represent a potential source of infection and dissemination of the agent.

Natural Modes of Infection

LCM and milder LCMV infections have been reported in Europe, the Americas, Australia, and Japan, and may occur wherever infected rodent hosts of the virus are found. Several serologic studies conducted in urban areas have shown that the prevalence of LCMV infection among humans ranges from 2% to 10%. Seroprevalence of 37.5% has been reported in humans in the Slovak Republic.[63]

The common house mouse, *Mus musculus*, naturally spreads LCMV. Once infected, these mice can become chronically infected as demonstrated by the presence of virus in blood and/or by persistently shedding virus in urine. Infections have also occurred in NHP in zoos, including macaques and marmosets. (*Callitrichid* hepatitis virus is a LCMV.)

Humans become infected by inhaling infectious aerosolized particles of rodent urine, feces, or saliva; by ingesting food contaminated with virus; by contamination of mucous membranes with infected body fluids; or by directly exposing cuts or other open wounds to virus-infected blood. Four recipients of organs from a donor who had unrecognized disseminated LCMV infection sustained severe disease and three succumbed. The source of donor infection was traced to a pet hamster that was not overtly ill.[64]

Laboratory Safety and Containment Recommendations

The agent may be present in blood, CSF, urine, secretions of the nasopharynx, feces and tissues of infected animal hosts and humans. Parenteral inoculation, inhalation, contamination of mucous membranes or broken skin with infectious tissues or fluids from infected animals are common hazards. Aerosol transmission is well documented.[60]

Of special note, tumors may acquire LCMV as an adventitious virus without obvious effects on the tumor. Virus may survive freezing and storage in liquid nitrogen for long periods. When infected tumor cells are transplanted,

subsequent infection of the host and virus excretion may ensue. Pregnant women infected with LCMV have transmitted the virus to their fetuses with death or serious central nervous system malformation as a consequence.[65]

BSL-2 practices, containment equipment, and facilities are suitable for activities utilizing known or potentially infectious body fluids, and for cell culture passage of laboratory-adapted strains. BSL-3 is required for activities with high potential for aerosol production, work with production quantities or high concentrations of infectious materials, and for manipulation of infected transplantable tumors, field isolates and clinical materials from human cases. Strains of LCMV that are shown to be lethal in non-human primates should be handled at BSL-3. ABSL-2 practices, containment equipment, and facilities are suitable for studies in adult mice with mouse brain-passaged strains requiring BSL-2 containment. Work with infected hamsters also should be done at ABSL-3.

Special Issues

Vaccines Vaccines are not available for use in humans.

Transfer of Agent Importation of this agent may require CDC and/or USDA 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.

Poliovirus

Poliovirus is the type species of the *Enterovirus* genus in the family *Picornaviridae.* Enteroviruses are transient inhabitants of the gastrointestinal tract, and are stable at acid pH. Picornaviruses are small, ether-insensitive viruses with an RNA genome.

There are three poliovirus serotypes (P1, P2, and P3). Immunity to one serotype does not produce significant immunity to the other serotypes.

Occupational Infections

Laboratory-associated poliomyelitis is uncommon. Twelve cases, including two deaths, were reported between 1941 and 1976.[62,66] No laboratory-associated poliomyelitis has been reported for nearly 30 years. Both inactivated poliovirus vaccine (IPV) and oral poliovirus vaccine (OPV) are highly effective in preventing disease, but neither vaccine provides complete protection against infection. Poliovirus infections among immunized laboratory workers are uncommon but remain undetermined in the absence of laboratory confirmation. An immunized laboratory worker may unknowingly be a source of poliovirus transmission to unvaccinated persons in the community.[67]

Natural Modes of Infection

At one time poliovirus infection occurred throughout the world. Transmission of wild poliovirus ceased in the United States in 1979, or possibly earlier. A polio eradication program conducted by the Pan American Health Organization led to elimination of polio from the Western Hemisphere in 1991. The Global Polio Eradication Program has dramatically reduced poliovirus transmission throughout the world.

Humans are the only known reservoir of poliovirus, which is transmitted most frequently by persons with unapparent infections. Person-to-person spread of poliovirus via the fecal-oral route is the most important route of transmission, although the oral-oral route may account for some cases.

Laboratory Safety and Containment Recommendations

The agent is present in the feces and in throat secretions of infected persons and in lymph nodes, brain tissue, and spinal cord tissue in fatal cases. For non-immunized persons in the laboratory, ingestion or parenteral inoculation are the primary routes of infection. For immunized persons, the primary risks are the same, except for parenteral inoculation, which likely presents a lower risk. The importance of aerosol exposure is unknown. Laboratory animal-associated infections have not been reported, but infected nonhuman primates should be considered to present a risk.

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing wild poliovirus infectious culture fluids, environmental samples, and clinical materials. In addition, potentially infectious materials collected for any purpose should be handled at BSL-2. Laboratory personnel working with such materials must have documented polio vaccination. Persons who have had a primary series of OPV or IPV and who are at an increased risk can receive another dose of IPV, but available data do not indicate the need for more than a single lifetime IPV booster dose for adults.[68] ABSL-2 practices, containment equipment, and facilities are recommended for studies of virulent viruses in animals. Laboratories should use authentic Sabin OPV attenuated strains unless there are strong scientific reasons for working with wild polioviruses.

In anticipation of polio eradication, the WHO recommends destruction of all poliovirus stocks and potential infectious materials if there is no longer a programmatic or research need for such materials.[69] Institutions/laboratories in the United States that currently retain wild poliovirus infectious or potential infectious material should be on the United States National Inventory maintained by CDC. When one year has elapsed after detection of the last wild poliovirus worldwide, CDC will inform relevant institutions/laboratories about additional containment procedures. Safety recommendations are subject to change based on international polio eradication activities.

Special Issues

When OPV immunization stops, global control and biosafety requirements for wild as well as attenuated (Sabin) poliovirus materials are expected to become more stringent, consistent with the increased consequences of inadvertent transmission to a growing susceptible community.

Transfer of Agent Importation of this agent may require CDC and/or USDA 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.

Poxviruses

Four genera of the subfamily *Chordopoxvirinae,* family *Poxviridae,* (*Orthopoxvirus*, *Parapoxvirus*, *Yatapoxvirus*, and *Molluscipoxvirus*) contain species that can cause lesions on human skin or mucous membranes with mild to severe systemic rash illness in laboratorians. Species within the first three genera mostly arise as zoonotic agents.[70,71] Laboratory-acquired poxvirus infections of most concern are from the orthopoxviruses that infect humans: variola virus (causes smallpox; human-specific), monkeypox virus (causes smallpox-like disease), *cowpox virus* (causes skin pustule, generalized rash), and vaccinia virus (causes skin pustule, systemic illness).[70-75]

Occupational Infections

Vaccinia virus, the leading agent of laboratory-acquired poxvirus infections, is used to make the current smallpox vaccine and may occur as a rare zoonosis.[70,71] Laboratory-acquired infections with standard, mutant, or bioengineered forms of vaccinia virus have occurred, even in previously vaccinated laboratorians. In addition, vaccination with live vaccinia virus sometimes has side effects, which range from mild events (e.g., fever, fatigue, swollen lymph nodes) to rare, severe, and at times fatal outcomes (e.g., generalized vaccinia, encephalitis, vaccinia necrosum, eczema vaccinatum, ocular keratitis, corneal infection, fetal infection of pregnancy, and possibly myocardial infarction, myopericarditis, or angina), thus vaccination contraindications should be carefully followed.[70,73-75]

Natural Modes of Infection

Smallpox has been eradicated from the world since 1980, but monkey pox virus is endemic in rodents in parts of Africa. Importation of African rodents into North America in 2003 resulted in an outbreak of monkeypox in humans.[72] Molluscum contagiosum, a disease due to *Molluscipoxvirus* infection, results in pearly white lesions that may persist for months in persons immunocompromised for various

reasons, including chronic illness, AIDS, other infections, medications, cancer and cancer therapies, or pregnancy.[70]

Laboratory Safety and Containment Recommendations

Poxviruses are stable in a wide range of environmental temperatures and humidity and may be transmitted by fomites.[70] Virus may enter the body through mucous membranes, broken skin, or by ingestion, parenteral inoculation or droplet or fine-particle aerosol inhalation. Sources of laboratory-acquired infection include exposure to aerosols, environmental samples, naturally or experimentally infected animals, infectious cultures, or clinical samples, including vesiculopustular rash lesion fluid or crusted scabs, various tissue specimens, excretions and respiratory secretions.

Worldwide, all live variola virus work is to be done only within WHO approved BSL-4/ABSL-4 facilities; one is at the CDC in Atlanta and the other is at the State Research Center of Virology and Biotechnology (VECTOR) in Koltsovo, Russia.[76]

In general, all persons working in or entering laboratory or animal care areas where activities with vaccinia, monkey pox, or cowpox viruses are being conducted should have evidence of satisfactory vaccination. Vaccination is advised every three years for work with monkeypox virus and every 10 years for cowpox and vaccinia viruses (neither vaccination nor vaccinia immunoglobulin protect against poxviruses of other genera).[73-75]

ABSL-3 practices, containment equipment, and facilities are recommended for monkeypox work in experimentally or naturally infected animals. BSL-2 facilities with BSL-3 practices are advised if vaccinated personnel perform other work with monkeypox virus. These practices include the use of Class I or II BSCs and barriers, such as safety cups or sealed rotors, for all centrifugations. The *NIH Guidelines* have assessed the risk of manipulating attenuated vaccinia strains (modified virus Ankara [MVA], NYVAC, TROVAC, and ALVAC) in areas where no other human orthopoxviruses are being used and have recommended BSL-1.[76] However, higher levels of containment are recommended if these strains are used in work areas where other orthopoxviruses are manipulated. Vaccination is not required for individuals working only in laboratories where no other orthopoxviruses or recombinants are handled.[75] BSL-2 and ABSL-2 plus vaccination are recommended for work with most other poxviruses.

Special Issues

Other Considerations The CDC Web site *www.cdc.gov* provides information on poxviruses, especially variola and monkeypox viruses, smallpox vaccination, and reporting vaccination adverse events. Clinical and other laboratories using poxviruses and clinicians can phone the CDC Clinician Information Line (877-554-4625) and/or the CDC public information hotline (888-246-2675) concerning variola and other human poxvirus infections, smallpox vaccine, vaccinia

immunoglobulin, poxvirus antiviral drugs, or other treatments or quarantine issues. Contact CDC regarding applications to transfer monkeypox viruses.

Transfer of Agent Importation of this agent may require CDC and/or USDA 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.

Rabies Virus (and related lyssaviruses)

Rabies is an acute, progressive, fatal encephalitis caused by negative-stranded RNA viruses in the genus *Lyssavirus*, family *Rhabdoviridae*.[77] *Rabies virus* is the representative member (type species) of the genus. Members of the group include Australian bat lyssavirus, Duvenhage virus, European bat lyssavirus[1], European bat lyssavirus[2], Lagos bat virus, and Mokola virus.

Occupational Infections

Rabies LAI are extremely rare; two have been documented. Both resulted from presumed exposure to high concentrations of infectious aerosols, one generated in a vaccine production facility,[78] and the other in a research facility.[79] Naturally or experimentally infected animals, their tissues, and their excretions are a potential source of exposure for laboratory and animal care personnel.

Natural Modes of Infection

The natural hosts of rabies are many bat species and terrestrial carnivores, but most mammals can be infected. The saliva of infected animals is highly infectious, and bites are the usual means of transmission, although infection through superficial skin lesions or mucosa is possible.

Laboratory Safety and Containment Recommendations

When working with infected animals, the highest viral concentrations are present in central nervous system (CNS) tissue, salivary glands, and saliva, but rabies viral antigens may be detected in all innervated tissues. The most likely sources for exposure of laboratory and animal care personnel are accidental parenteral inoculation, cuts, or needle sticks with contaminated laboratory equipment, bites by infected animals, and exposure of mucous membranes or broken skin to infectious tissue or fluids. Infectious aerosols have not been a demonstrated hazard to personnel working with routine clinical materials or conducting diagnostic examinations. Fixed and attenuated strains of virus are presumed to be less hazardous, but the two recorded cases of laboratory-associated rabies resulted from presumed exposure to the fixed Challenge Virus Standard and Street Alabama Dufferin strains, respectively.

BSL-2 and/or ABSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials or animals. Pre-exposure rabies vaccination is recommended for all individuals prior to working with lyssaviruses or infected animals, or engaging in diagnostic, production, or research activities with these viruses.[80] Rabies vaccination also is recommended for all individuals entering or working in the same room where lyssaviruses or infected animals are used. Prompt administration of postexposure booster vaccinations is recommended following recognized exposures in previously vaccinated individuals per current guidelines.[81] For routine diagnostic activities, it is not always feasible to open the skull or remove the brain of an infected animal within a BSC, but it is pertinent to use appropriate methods and personal protection equipment, including dedicated laboratory clothing, heavy protective gloves to avoid cuts or sticks from cutting instruments or bone fragments, and a face shield or PAPR to protect the skin and mucous membranes of the eyes, nose, and mouth from exposure to tissue fragments or infectious droplets.

If a Stryker saw is used to open the skull, avoid contacting brain tissue with the blade of the saw. Additional primary containment and personnel precautions, such as those described for BSL-3, are indicated for activities with a high potential for droplet or aerosol production, and for activities involving large production quantities or high concentrations of infectious materials.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA 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.

Retroviruses, including Human and Simian Immunodeficiency Viruses (HIV and SIV)

The family *Retroviridae* is divided into two subfamilies, the *Orthoretrovirinae* with six genera including the Lentivirus genus, which includes HIV-1 and HIV-2. Other important human pathogens are human T-lymphotropic viruses 1 and 2 (HTLV-1 and HTLV-2), members of the Deltaretrovirus genus. The Spumaretrovirinae, with one genus, Spumavirus, contains a variety of NHP viruses (foamy viruses) that can occasionally infect humans in close contact with NHPs**.**

Occupational Infections

Data on occupational HIV transmission in laboratory workers are collected through two CDC-supported national surveillance systems: surveillance for 1) AIDS, and 2) HIV-infected persons who may have acquired their infection through occupational exposures. For surveillance purposes, laboratory workers are defined as those persons, including students and trainees, who have worked in a clinical or HIV laboratory setting anytime since 1978. Cases reported in these two systems are classified as either documented or possible occupational transmission. Those classified as documented occupational transmission had evidence of HIV seroconversion (a negative HIV-antibody test at the time of the exposure which converted to positive) following a discrete percutaneous or mucocutaneous occupational exposure to blood, body fluids, or other clinical or laboratory specimens. As of June 1998, CDC had reports of 16 laboratory workers (all clinical) in the United States with documented occupational transmission.[82]

Workers have been reported to develop antibodies to simian immunodeficiency virus (SIV) following exposures. One case was associated with a needle-stick that occurred while the worker was manipulating a blood-contaminated needle after bleeding an SIV-infected macaque monkey.[83] Another case involved a laboratory worker who handled macaque SIV-infected blood specimens without gloves. Though no specific incident was recalled, this worker had dermatitis on the forearms and hands while working with the infected blood specimens.[84] A third worker[85] was exposed to SIV-infected primate blood through a needle-stick and subsequently developed antibodies to SIV. To date there is no evidence of illness or immunological incompetence in any of these workers.

Natural Modes of Infection

Retroviruses are widely distributed as infectious agents of vertebrates. Within the human population, spread is by close sexual contact or parenteral exposure through blood or blood products.

Laboratory Safety and Containment Recommendations

HIV has been isolated from blood, semen, saliva, tears, urine, CSF, amniotic fluid, breast milk, cervical secretion, and tissues of infected persons and experimentally infected nonhuman primates.[86]

Although the risk of occupationally-acquired HIV is primarily through exposure to infected blood, it is also prudent to wear gloves when manipulating other body fluids such as feces, saliva, urine, tears, sweat, vomitus, and human breast milk. This also reduces the potential for exposure to other microorganisms that may cause other types of infections.

In the laboratory, virus should be presumed to be present in all blood or clinical specimens contaminated with blood, in any unfixed tissue or organ (other

than intact skin) from a human (living or dead), in HIV cultures, in all materials derived from HIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.

SIV has been isolated from blood, CSF, and a variety of tissues of infected nonhuman primates. Limited data exist on the concentration of virus in semen, saliva, cervical secretions, urine, breast milk, and amniotic fluid. Virus should be presumed to be present in all SIV cultures, in animals experimentally infected or inoculated with SIV, in all materials derived from SIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.[87]

The skin (especially when scratches, cuts, abrasions, dermatitis, or other lesions are present) and mucous membranes of the eye, nose, and mouth should be considered as potential pathways for entry of these retroviruses during laboratory activities. It is unknown whether infection can occur via the respiratory tract. The need for using sharps in the laboratory should be evaluated. Needles, sharp instruments, broken glass, and other sharp objects must be carefully handled and properly discarded. Care must be taken to avoid spilling and splashing infected cell-culture liquid and other potentially infected materials.[85]

BSL-2 practices, containment equipment, and facilities are recommended for activities involving blood-contaminated clinical specimens, body fluids and tissues. HTLV-1 and HTLV-2 should also be handled at this level. Activities such as producing research-laboratory-scale quantities of HIV or SIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, are performed in a BSL-2 facility, using BSL-3 practices. Activities involving large-scale volumes or preparation of concentrated HIV or SIV are conducted at BSL-3. ABSL-2 is appropriate for NHP and other animals infected with HIV or SIV. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BSL-2.

In addition to the aforementioned recommendations, persons working with HIV, SIV, or other bloodborne pathogens should consult the OSHA Bloodborne Pathogen Standard.[88] Questions related to interpretation of this Standard should be directed to federal, regional or state OSHA offices.

Special Issues

It is recommended that all institutions establish written policies regarding the management of laboratory exposure to HIV and SIV, including treatment and prophylaxis protocols. (See Section VII.)

The risk associated with retroviral vector systems can vary significantly, especially lentiviral vectors. Because the risk associated with each gene transfer system can vary, no specific guideline can be offered other than to have all gene transfer protocols reviewed by an IBC.

Transfer of Agent Importation of this agent may require CDC and/or USDA 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.

Severe Acute Respiratory Syndrome (SARS) Coronavirus

SARS is a viral respiratory illness caused by a previously undescribed coronavirus, SARS-associated coronavirus (SARS-CoV) within the family *Coronaviridae.* SARS was retrospectively recognized in China in November 2002. Over the next few months, the illness spread to other south-east.

Asian countries, North America, South America, and Europe following major airline routes. The majority of disease spread occurred in hospitals, among family members and contacts of hospital workers. From November 2002 through July 2003, when the global outbreak was contained, a total of 8,098 probable cases of SARS were reported to the WHO from 29 countries.[89]

In general, SARS patients present with fever (temperature greater than 100.4°F [>38.0°C]), malaise and myalgias quickly followed by respiratory symptoms including shortness of breath and cough. Ten to 20 percent of patients may have diarrhea. Review of probable cases indicates that the shortness of breath sometimes rapidly progresses to respiratory failure requiring ventilation. The case fatality rate is about 11%.

Occupational Infections

Healthcare workers are at increased risk of acquiring SARS from an infected patient especially if involved in pulmonary/respiratory procedures such as endotracheal intubation, aerosolization or nebulization of medications, diagnostic sputum induction, airway suctioning, positive pressure ventilation and high-frequency oscillatory ventilation.

Two confirmed episodes of SARS-CoV transmission to laboratory workers occurred in research laboratories in Singapore and Taiwan.[89,90] Both occurrences were linked to breaches in laboratory practices. Laboratory-acquired infections in China during 2004 demonstrated secondary and tertiary spread of the disease to close contacts and healthcare providers of one of the employees involved.[91] Although no laboratory-acquired cases have been associated with the routine processing of diagnostic specimens, SARS coronavirus represents an emerging infectious disease for which risk to the medical and laboratory community is not fully understood.

Natural Modes of Infection

The mode of transmission in nature is not well understood. It appears that SARS is transmitted from person-to-person through close contact such as caring for, living with, or having direct contact with respiratory secretions or body fluids of a suspect or probable case.[92] SARS is thought to be spread primarily through droplets, aerosols and possibly fomites. The natural reservoir for SARS CoV is unknown.

Laboratory Safety and Containment Recommendations

SARS-CoV may be detected in respiratory, blood, or stool specimens. The exact mode of transmission of SARS-CoV laboratory-acquired infection has not been established, but in clinical settings the primary mode of transmission appears through direct or indirect contact of mucous membranes with infectious respiratory droplets.[93,94]

In clinical laboratories, whole blood, serum, plasma and urine specimens should be handled using Standard Precautions, which includes use of gloves, gown, mask, and eye protection. Any procedure with the potential to generate aerosols (e.g., vortexing or sonication of specimens in an open tube) should be performed in a BSC. Use sealed centrifuge rotors or gasketed safety carriers for centrifugation. Rotors and safety carriers should be loaded and unloaded in a BSC. Procedures conducted outside a BSC must be performed in a manner that minimizes the risk of personnel exposure and environmental release.

The following procedures may be conducted in the BSL-2 setting: pathologic examination and processing of formalin-fixed or otherwise inactivated tissues, molecular analysis of extracted nucleic acid preparations, electron microscopic studies with glutaraldehyde-fixed grids, routine examination of bacterial and fungal cultures, routine staining and microscopic analysis of fixed smears, and final packaging of specimens for transport to diagnostic laboratories for additional testing (specimens should already be in a sealed, decontaminated primary container).

Activities involving manipulation of untreated specimens should be performed in BSL-2 facilities following BSL-3 practices. In the rare event that a procedure or process involving untreated specimens cannot be conducted in a BSC, gloves, gown, eye protection, and respiratory protection (acceptable methods of respiratory protection include: a properly fit-tested, National Institute for Occupational Safety and Health [NIOSH]-approved filter respirator [N-95 or higher level] or a PAPR equipped with HEPA filters) should be used. All personnel who use respiratory protective devices should be enrolled in an appropriately constituted respiratory protection program.

Work surfaces should be decontaminated upon completion of work with appropriate disinfectants. All waste must be decontaminated prior to disposal.

SARS-CoV propagation in cell culture and the initial characterization of viral agents recovered in cultures of SARS specimens must be performed in a BSL-3 facility using BSL-3 practices and procedures. Risk assessment may dictate the additional use of respiratory protection.

Inoculation of animals for potential recovery of SARS-CoV from SARS samples, research studies, and protocols involving animal inoculation for characterization of putative SARS agents must be performed in ABSL-3 facilities using ABSL-3 work practices. Respiratory protection should be used as warranted by risk assessment.

In the event of any break in laboratory procedure or accidents (e.g., accidental spillage of material suspected of containing SARS-CoV), procedures for emergency exposure management and/or environmental decontamination should be immediately implemented and the supervisor should be notified. The worker and the supervisor, in consultation with occupational health or infection control personnel, should evaluate the break in procedure to determine if an exposure occurred (see Special Issues, below).

Special Issues

Occupational Health Considerations Institutions performing work with SARS coronavirus should require storage of a baseline serum sample from individuals who work with the virus or virus-containing specimens. Personnel working with the virus or samples containing or potentially containing the virus should be trained regarding the symptoms of SARS-CoV infection and counseled to report any fever or respiratory symptoms to their supervisor immediately. They should be evaluated for possible exposure and the clinical features and course of their illness should be closely monitored. Institutions performing work with the SARS-CoV or handling specimens likely to contain the agent should develop and implement a specific occupational medical plan with respect to this agent. The plan, at a minimum, should contain procedures for managing:

- identifiable breaks in laboratory procedures;
- exposed workers without symptoms;
- exposed workers who develop symptoms within ten days of an exposure; and
- symptomatic laboratory workers with no recognized exposure.

Further information and guidance regarding the development of a personnel exposure response plan is available from the CDC.[95] Laboratory workers who are believed to have had a laboratory exposure to SARS-CoV should be evaluated, counseled about the risk of SARS-CoV transmission to others, and monitored for fever or lower respiratory symptoms as well as for any of the following: sore throat, rhinorrhea, chills, rigors, myalgia, headache, and diarrhea.

Local and/or state public health departments should be promptly notified of laboratory exposures and illness in exposed laboratory workers.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

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Section VIII-F: Arboviruses and Related Zoonotic Viruses

In 1979, the American Committee on Arthropod-Borne Viruses (ACAV) Subcommittee on Arbovirus Laboratory Safety (SALS) first provided biosafety recommendations for each of the 424 viruses then registered in the International Catalogue of Arboviruses, including Certain Other Viruses of Vertebrates.[1] Working together, SALS, the CDC and the NIH have periodically updated the catalogue by providing recommended biosafety practices and containment for arboviruses registered since 1979. These recommendations are based, in part, on risk assessments derived from information provided by a worldwide survey of laboratories working with arboviruses, new published reports on the viruses, as well as discussions with scientists working with each virus.

Table 6, located at the end of this Section, provides an alphabetical listing of 597 viruses and includes common name, virus family or genus, acronym, BSL recommendation, the basis for the rating, the antigenic group[2] (if known), HEPA filtration requirements, and regulatory requirements (i.e., import/export permits from either the CDC or the USDA). In addition, many of the organisms are classified as select agents and require special security measures to possess, use, or transport. (See Appendix F.) Table 4 provides a key for the SALS basis for assignment of viruses listed in Table 6.

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
- the organism presented a significant risk of laboratory-acquired infection.

These recommendations were made in August 2005; 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 the USDA for additional biosafety requirements. USDA guidance may supersede the information found in this Chapter.

Recommendations for the containment of infected arthropod vectors were drafted by a subcommittee of the American Committee on Medical Entomology (ACME), and circulated widely among medical entomology professionals. (See Appendix E.)

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 (Table 5). The agents in Table 4 and 5 may require permits from USDA/DOC/DHHS.

Table 4. Explanation of Symbols Used in Table 6 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	Disease in sheep, cattle or horses.
A2	Fatal human laboratory infection—probably aerosol.
A3	Extensive laboratory experience and mild nature of aerosol laboratory infections justifies BSL-2.
A4	Placed in BSL-4 based on the close antigenic relationship with a known BSL-4 agent plus insufficient experience.
A5	BSL-2 arenaviruses are not known to cause serious acute disease in humans and are not acutely pathogenic for laboratory animals including primates. In view of reported high frequency of laboratory aerosol infection in workers manipulating high concentrations of Pichinde virus, it is strongly recommended that work with high concentrations of BSL-2 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 3 or more viruses, all of which are classified at this level.
A8	BSL-2 hantaviruses 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 or experimentally infected rodents be conducted at BSL-3.

Table 5. Vaccine Strains of BSL-3 and BSL-4 Viruses that May Be Handled as BSL-2

Virus	Vaccine Strain
Chikungunya	181/25
Junin	Candid #1
Rift Valley fever	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.

Viruses with BSL-2 Containment Recommended

The recommendation for conducting work with the viruses listed in Table 6 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 a) no overt laboratory-associated infections are reported, b) infections resulted from exposures other than by infectious aerosols, or c) 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 comprise accidental parenteral inoculation, contact of the virus with broken skin or mucous membranes, and bites of infected laboratory rodents or arthropods. Properly maintained BSCs, preferable Class II, or other appropriate personal protective equipment 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, embryonate hen’s eggs, and rodents.

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

Viruses with BSL-3 Containment Recommended

The recommendations for viruses listed in Table 6 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 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, 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 those entering rooms where the agents or infected animals are present.

Junin virus has been reclassified to BSL-3, 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 (CETBE) viruses to BSL-3, provided all at-risk personnel are immunized. CETBE is not a registered name in *The International Catalogue of Arboviruses* (1985). Until the registration issue is resolved taxonomically, CETBE 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 CETBE group of genetically (>98%) homogeneous viruses, the efficacy of this vaccine against Russian spring-summer encephalitis (RSSE) virus infections has not been established. Thus, the CETBE group of viruses has been reclassified as BSL-3 when personnel are immunized with CETBE vaccine, while RSSE remains classified as BSL-4. It should be noted that CETBE viruses are currently listed as select agents and require special security and permitting considerations. (See Appendix F.)

Investigational vaccines for eastern equine encephalomyelitis (EEE) virus, Venezuelan equine encephalitis (VEE), western equine encephalomyelitis (WEE) virus, 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. Details are available at the end of this section.

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

If the investigational vaccine is contraindicated, does not provide acceptable reliability for producing an immune response, or laboratory personnel refuse vaccination, the use of appropriate personal protective equipment may provide an alternative. Respiratory protection, such as use of a PAPR, should be considered in areas 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 accordance with the institution's respiratory protection program. Other degrees of respiratory protection may be warranted based on an assessment of risk as defined in Chapter 2 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 under BSL-2 and BSL-3 requirements must be continually and strictly reinforced, regardless of whether investigational vaccines are used.

Non-licensed vaccines are available in limited quantities and administered on-site at the Special Immunization Program of USAMRIID. IND vaccines are administered under a cooperative agreement between the U.S. Army and the individual's requesting organization. Contact the Special Immunization Program by telephone at (301) 619-4653.

Enhanced BSL-3 Containment

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 might include: 1) enhanced respiratory protection of personnel against aerosols; 2) HEPA filtration of dedicated exhaust air from the laboratory; and 3) personal body shower. Additional appropriate training for all animal care personnel should be considered.

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 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, and in arthropods, rodents, and 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.[3,4]

BSL-4 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials of human, animal, or arthropod origin. 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 maximum containment facility.[5]

Dealing with Unknown Arboviruses

The ACAV has published reports documenting laboratory workers who acquired arbovirus infections during the course of their duties.[6] In the first such document, 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 laboratory-acquired infections 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.[7,8]

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 laboratory-acquired infections 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 BSC while wearing extensive personal protective equipment in contrast to working with viruses on an open bench top) and significant changes in laboratory equipment and facilities (e.g., BSC, PAPR) available since the initial SALS evaluation. Clearly, when dealing with a newly recognized arbovirus, there is insufficient previous experience with it; thus, the virus should be assigned a higher biosafety level. However, with increased ability to safely characterize viruses, the relationship to other disease-causing arboviruses can be established with reduced exposure to the investigators. Therefore, in addition to those established by SALS, additional assessment criteria should be considered.

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.[9] 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. 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, assignment to a different biosafety level may be justified.[10] A clear description of the strains involved should accompany any risk assessment.

Most of the identified arboviruses have been assigned biosafety levels; however, a number of those that are infrequently studied, newly identified, or have only single isolation events may not have been evaluated by SALS, ACAV, CDC, or the NIH (Table 6). Thorough risk assessment is important for all arboviral research and it is of particular importance for work involving unclassified viruses. A careful assessment by the laboratory director, institutional biosafety officer and safety committee, and as necessary, outside experts is necessary 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.[11-14]

Because chimeric viruses contain portions of multiple viruses, the IBC, 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;[15] 2) altered virulence characteristics or attenuation compared with the parental viruses in animal models;[16] 3) virulence or attenuation patterns by intracranial routes using large doses for agents affecting the CNS;[17,18] and 4) demonstration of lack of reversion to virulence or parental phenotype.

Many patterns of attenuation have been observed with chimeric flaviviruses and alphaviruses using the criteria described above. Additionally, some of these chimeras are in phase II testing as human vaccines.[19]

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 limited possibility of laboratory recombination to 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 rigorouscontainment requirements of the parental strains. The local IBC 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 Biotechnology Activities and/or the Recombinant DNA Advisory Committee (RAC) may be necessary.

West Nile Virus (WNV)

WNV has emerged in recent years in temperate regions of Europe and North America, presenting a threat to public and animal health. 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.[20] The ecology was characterized in Egypt in the 1950s; equine disease was first noted in Egypt and France in the early 1960s.[21,22] It first appeared in North America in 1999 as encephalitis reported in humans and horses.[23] The virus has been detected in Africa, Europe, the Middle East, west and central Asia, Oceania (subtype Kunjin virus), and most recently, North America.

Occupational Infections

LAI with WNV have been reported in the literature. SALS reported 15 human infections from laboratory accidents in 1980. One of these infections was attributed to aerosol exposure. Two parenteral inoculations have been reported recently during work with animals.[24]

Natural Modes of Infections

In the United States, 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. People, 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 may be at higher risk of infection.

BSL-2 practices, containment equipment, and facilities are recommended for activities with human diagnostic specimens, although it is unusual to recover virus from specimens obtained from clinically ill patients. BSL-2 is recommended for processing field collected mosquito pools whereas BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of WNV cultures and for experimental animal and vector studies, respectively.

Dissection of field collected dead birds for histopathology and culture is recommended at BSL-3 containment due to the potentially high levels of virus found in such samples. Non-invasive procedures performed on dead birds (such as oropharyngeal or cloacal swabs) can be conducted at BSL-2.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA 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 (EEE) Virus, Venezuelan Equine Encephalitis (VEE) Virus, and Western Equine Encephalitis (WEE) Virus

VEE, EEE, and WEE viruses 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. Incubation period ranges from 1-10 days and the duration of acute illness is typically days to weeks depending upon severity of illness. Although not the natural route of transmission, the viruses are highly infectious by the aerosol route; laboratory acquired infections have been documented.[25]

Occupational Infections

These alphaviruses, especially VEE virus, are infectious by aerosol in laboratory studies and more than 160 EEE virus, VEE virus, or WEE virus laboratory-acquired infections 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 EEE virus and WEE virus) and mosquitoes also are 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.

EEE virus occurs in focal locations along the eastern seaboard, the Gulf Coast and some inland Midwestern locations of the United States, in Canada, some Caribbean Islands, and Central and South America.[26] 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, while endemic foci of Bijou Bridge virus (VEE antigenic complex subtype III-B virus), have been described in the western United States.[27]

The WEE virus 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 mucus 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 under BSL-3 practices, containment equipment, and facilities. Due to the high risk of aerosol infection, additional personal protective equipment, including respiratory protection, should be considered for non-immune personnel. Animal work with VEE virus, EEE virus and WEE virus should be performed under ABSL-3 conditions. HEPA filtration is required on the exhaust system of laboratory and animal facilities using VEE virus.

Special Issues

Vaccines Two strains of VEE virus (TC-83 and V3526) are highly attenuated in vertebrate studies and have been either exempted (strain TC-83) or excluded (strain V3526) 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.

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 in lieu of vaccination. For personnel who have no neutralizing antibody titer (either by previous vaccination or natural infection), additional respiratory protection is recommended for all procedures.

Select Agent VEE virus and EEE virus 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 this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

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.[28] 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 in 1977 to 1979 in Egypt with many thousands of human cases and 610 reported deaths.[29]

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 occurs. Hemorrhagic fever develops as the primary illness proceeds and is characterized by disseminated intravascular coagulation and hepatitis. Perhaps 2% of cases will develop this complication and the mortality is high. Encephalitis follows an 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 an 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.[30,31]

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.[32] 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; as yet undefined native African vertebrate amplifier is thought to exist and very high viremias in humans are thought to play some role in viral amplifications.[33]

Transmission of diseases occurs between infected animals but is of low efficiency and 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 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 a RVFV laboratory and subsequently removed.

Diagnostic or research studies outside endemic areas should be performed in a BSL-3 laboratory. Personnel also must have additional respiratory protection (such as a PAPR) or be vaccinated for RVFV. In addition, the USDA may require

full BSL-3-Ag containment for research conducted in non-endemic areas in loose-housed animals. (See Appendix D.)

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 field workers under investigational protocols, but neither vaccine is available at this time.

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

The live-attenuated MP-12 vaccine strain is specifically exempted from the Select Agent rules. In general, BSL-2 containment is recommended for working with this strain.

The USDA may require enhanced ABSL-3, ABSL-3, or BSL-3-Ag facilities and practices for working with RVFV in the United States. (See Appendix D.) Investigators should contact the USDA for further guidance before initiating research.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS.

Table 6. Alphabetic Listing of 597 Arboviruses and Hemorrhagic Fever Viruses*

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Abras	ABRV	*Orthobunvavarus*	2	A7	Patois	No
Absettarov	ABSV	*Flavivirus*	4	A4	B[f]	Yes
Abu Hammad	AHV	*Nairovirus*	2	S	Dera Ghazi Khan	No
Acado	ACDV	*Orbivirus*	2	S	Corriparta	No
Acara	ACAV	*Orthobunyavirus*	2	S	Capim	No
Adelaide River	ARV	*Lyssavirus*	2	IE	Bovine Ephemeral Fever	No
African Horse sickness	AHSV	*Orbivirus*	3[c]	A1	African Horsesickness	Yes
African Swine Fever	ASFV	*Asfivirus*	3[c]	IE	Asfivirus	Yes

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Aguacate	AGUV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Aino	AINOV	*Orthobunyavirus*	2	S	Simbu	No
Akabane	AKAV	*Orthobunyavirus*	3[c]	S	Simbu	Yes
Alenquer	ALEV	*Phlebovirus*	2	IE	Phlebotomus Fever	No
Alfuy	ALFV	*Flavivirus*	2	S	B[f]	No
Alkhumra	ALKV	*Flavivirus*	4	A4	B[f]	Yes
Allpahuayo	ALLPV	*Arenavirus*	3	IE	Tacaribe	No
Almeirim	ALMV	*Orbivirus*	2	IE	Changuinola	No
Almpiwar	ALMV	*Rhabdoviridae*	2	S		No
Altamira	ALTV	*Orbivirus*	2	IE	Changuinola	No
Amapari	AMAV	*Arenavirus*	2	A5	Tacaribe	No
Ambe	AMBEV	*Phlebovirus*	2	IE		No
Ananindeua	ANUV	*Orthobunyavirus*	2	A7	Guama	No
Andasibe	ANDV	*Orbivirus*	2	A7		No
Andes	ANDV	*Hantavirus*	3[a]	IE	Hantaan	No
Anhanga	ANHV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Anhembi	AMBV	*Orthobunyavirus*	2	S	Bunyamwera	No
Anopheles A	ANAV	*Orthobunyavirus*	2	S	Anopheles A	No
Anopheles B	ANBV	*Orthobunyavirus*	2	S	Anopheles B	No
Antequera	ANTV	*Bunyaviridae*	2	IE	Resistencia	No
Apeu	APEUV	*Orthobunyavirus*	2	S	C[f]	No
Apoi	APOIV	*Flavivirus*	2	S	B[f]	No
Araguari	ARAV	Unassigned	3	IE		No
Aransas Bay	ABV	*Bunyaviridae*	2	IE	UPOLU	No
Arbia	ARBV	*Phlebovirus*	2	IE	Phlebotomus Fever	No
Arboledas	ADSV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Aride	ARIV	*Unassigned*	2	S		No
Ariquemes	ARQV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Arkonam	ARKV	*Orbivirus*	2	S	Ieri	No
Armero	ARMV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Aroa	AROAV	*Flavivirus*	2	S	B[f]	No
Aruac	ARUV	*Rhabdoviridae*	2	S		No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Arumateua	ARMTV	*Orthobunyavirus*	2	A7		No
Arumowot	AMTV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Aura	AURAV	*Alphavirus*	2	S	A[f]	No
Avalon	AVAV	*Nairovirus*	2	S	Sakhalin	No
Babahoyo	BABV	*Orthobunyavirus*	2	A7	Patois	No
Babanki	BBKV	*Alphavirus*	2	A7	A[f]	No
Bagaza	BAGV	*Flavivirus*	2	S	B[f]	No
Bahig	BAHV	*Orthobunyavirus*	2	S	Tete	No
Bakau	BAKV	*Orthobunyavirus*	2	S	Bakau	No
Baku	BAKUV	*Orbivirus*	2	S	Kemerovo	No
Bandia	BDAV	*Nairovirus*	2	S	Qalyub	No
Bangoran	BGNV	*Rhabdoviridae*	2	S		No
Bangui	BGIV	*Bunyaviridae*	2	S		No
Banzi	BANV	*Flavivirus*	2	S	B[f]	No
Barmah Forest	BFV	*Alphavirus*	2	A7	A[f]	No
Barranqueras	BQSV	*Bunyaviridae*	2	IE	Resistencia	No
Barur	BARV	*Rhabdoviridae*	2	S	Kern Canyon	No
Batai	BATV	*Orthobunyavirus*	2	S	Bunyamwera	No
Batama	BMAV	*Orthobunyavirus*	2	A7	Tete	No
Batken	BKNV	*Thogotovirus*	2	IE		No
Bauline	BAUV	*Orbivirus*	2	S	Kemerovo	No
Bear Canyon	BRCV	*Arenavirus*	3	A7		No
Bebaru	BEBV	*Alphavirus*	2	S	A[f]	No
Belem	BLMV	*Bunyaviridae*	2	IE		No
Belmont	ELV	*Bunyaviridae*	2	S		No
Belterra	BELTV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Benevides	BENV	*Orthobunyavirus*	2	A7	Capim	No
Benfica	BENV	*Orthobunyavirus*	2	A7	Capim	No
Bermejo	BMJV	*Hantavirus*	3	IE	Hantaan	No
Berrimah	BRMV	*Lyssavirus*	2	IE	Bovine Ephemeral Fever	No
Beritoga	BERV	*Orthobunyavirus*	2	S	Guama	No
Bhanja	BHAV	*Bunyaviridae*	3	S	Bhanja	No
Bimbo	BBOV	*Rhabdoviridae*	2	IE		No

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Bimitti	BIMV	*Orthobunyavirus*	2	S	Guama	No
Birao	BIRV	*Orthobunyavirus*	2	S	Bunyamwera	No
Bluetoungue (exotic serotypes)	BTV	*Orbivirus*	3[c]	S	Bluetongue	No
Bluetoungue (non-exotic)	BTV	*Orbivirus*	2[c]	S	Bluetongue	No
Bobaya	BOBV	*Bunyaviridae*	2	IE		No
Bobia	BIAV	*Orthobunyavirus*	2	IE	Olifantsylei	No
Boraceia	BORV	*Orthobunyavirus*	2	S	Anopheles B	No
Botambi	BOTV	*Orthobunyavirus*	2	S	Olifantsylei	No
Boteke	BTKV	*Vesiculovirus*	2	S	Vesicular Stomatitis	No
Bouboui	BOUV	*Flavivirus*	2	S	B[f]	No
Bovine Ephemeral Fever	BEFV	*Lyssavirus*	3[c]	A1	Bovine Ephemeral Fever	No
Bozo	BOZOV	*Orthobunyavirus*	2	A7	Bunyamwera	No
Breu Branco	BRBV	*Orbivirus*	2	A7		No
Buenaventura	BUEV	*Phlebovirus*	2	IE	Phlebotomus Fever	No
Bujaru	BUJV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Bunyamwera	BUNV	*Orthobunyavirus*	2	S	Bunyamwera	No
Bunyip Creek	BCV	*Orbivirus*	2	S	Palyam	No
Burg El Arab	BEAV	*Rhabdoviridae*	2	S	Matariva	No
Bushbush	BSBV	*Orthobunyavirus*	2	S	Capim	No
Bussuquara	BSQV	*Flavivirus*	2	S	B[f]	No
Buttonwillow	BUTV	*Orthobunyavirus*	2	S	Simbu	No
Bwamba	BWAV	*Orthobunyavirus*	2	S	Bwamba	No
Cabassou	CABV	*Alphavirus*	3	IE	A[f]	Yes
Cacao	CACV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Cache Valley	CVV	*Orthobunyavirus*	2	S	Bunyamwera	No
Cacipacore	CPCV	*Flavivirus*	2	IE	B[f]	No
Caimito	CAIV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Calchaqui	CQIV	*Vesiculovirus*	2	A7	Vesicular Stomatitis	No
California Encephalitis	CEV	*Orthobunyavirus*	2	S	California	No
Calovo	CVOV	*Orthobunyavirus*	2	S	Bunyamwera	No
Cananeia	CNAV	*Orthobunyavirus*	2	IE	GUAMA	No

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Candiru	CDUV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Caninde	CANV	*Orbivirus*	2	IE	Changuinola	No
Cano Delgadito	CADV	*Hantavirus*	3[a]	IE	Hantaan	No
Cape Wrath	CWV	*Orbivirus*	2	S	Kemerovo	No
Capim	CAPV	*Orthobunyavirus*	2	S	Capim	No
Caraipe	CRPV	*Orthobunyavirus*	2	A7		No
Carajas	CRJV	*Vesiculovirus*	2	A7	Vesicular Stomatitis	No
Caraparu	CARV	*Orthobunyavirus*	2	S	C[f]	No
Carey Island	CIV	*Flavivirus*	2	S	B[f]	No
Catu	CATUV	*Orthobunyavirus*	2	S	Guama	No
Chaco	CHOV	*Rhabdoviridae*	2	S	Timbo	No
Chagres	CHGV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Chandipura	CHPV	*Vesiculovirus*	2	S	Vesicular Stomatitis	No
Changuinola	CGLV	*Orbivirus*	2	S	Changuinola	No
Charleville	CHVV	*Lyssavirus*	2	S	Rab	No
Chenuda	CNUV	*Orbivirus*	2	S	Kmerovo	No
Chikungunya	CHIKV	*Alphavirus*	3	S	A[f]	Yes
Chilibre	CHIV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Chim	CHIMV	*Bunyaviridae*	2	IE		No
Chobar Gorge	CGV	*Orbivirus*	2	S	Chobar Gorge	No
Clo Mor	CMV	*Nairovirus*	2	S	Sakhalin	No
Coastal Plains	CPV	*Lyssavirus*	2	IE	Tibrogargan	No
Cocal	COCV	*Vesiculovirus*	2	A3	Vesicular Stomatitis	No
Codajas	CDJV	*Orbivirus*	2	A7		No
Colorado Tick Fever	CTFV	*Coltivirus*	2	S	Colorado Tick Fever	No
Congo-Crimean Hemorrhagic Fever	CCHFV	*Nairovirus*	4	A6	CCHF	Yes
Connecticut	CNTV	*Rhabdoviridae*	2	IE	Sawgrass	No
Corfou	CFUV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Corriparta	CORV	*Orbivirus*	2	S	Corriaparta	No
Cotia	CPV	*Poxviridae*	2	S		No
Cowbone Ridge	CRV	*Flavivirus*	2	S	B[f]	No

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Csiro Village	CVGV	*Orbivirus*	2	S	Palyam	No
Cuiaba	CUIV	*Rhabdoviridae*	2	S		No
Curionopolis	CRNPV	*Rhabdoviridae*	2	A7		No
Dabakala	DABV	*Orthobunyavirus*	2	A7	Olifantsylei	No
D'Aguilar	DAGV	*Orbivirus*	2	S	Palyam	No
Dakar Bat Virus	DBV	*Flavivirus*	2	S	B[f]	No
Deer Tick Virus	DRTV	*Flavivirus*	3	A7		No
Dengue Virus Type 1	DENV-1	*Flavivirus*	2	S	B[f]	No
Dengue Virus Type 2	DENV-2	*Flavivirus*	2	S	B[f]	No
Dengue Virus Type 3	DENV-3	*Flavivirus*	2	S	B[f]	No
Dengue Virus Type 4	DENV-4	*Flavivirus*	2	S	B[f]	No
Dera Ghazi Khan	DGKV	*Nairovirus*	2	S	Dera Ghazi Khan	No
Dobrava-Belgrade	DOBV	*Hantavirus*	3[a]	IE		No
Dhori	DHOV	*Orthomyxoviridae*	2	S		No
Douglas	DOUV	*Orthobunyavirus*	3	IE	Simbu	No
Durania	DURV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Dugbe	DUGV	*Nairovirus*	3	S	Nairobi Sheep Disease	No
Eastern Equine Encephalitis	EEEV	*Alphavirus*	3[c]	S	A[f]	No
Ebola (Including Reston)	EBOV	*Filovirus*	4	S	EBO	Yes
Edge Hill	EHV	*Flavivirus*	2	S	B[f]	No
Enseada	ENSV	*Bunyaviridae*	3	IE		No
Entebbe Bat	ENTV	*Flavivirus*	2	S	B[f]	No
Epizootic Hemorrhagic Disease	EHDV	*Orbivirus*	2	S	Epizootic Hemorrhagic Disease	No
Erve	ERVEV	*Bunyaviridae*	2	S	Thiafora	No
Estero Real	ERV	*Orthobunyavirus*	2	IE	Patois	No
Eubenangee	EUBV	*Orbivirus*	2	S	Eubenangee	No
Everglades	EVEV	*Alphavirus*	3	S	A[f]	Yes
Eyach	EYAV	*Coltivirus*	2	S	Colorado Tick Fever	No
Farmington	FRMV	*Vesiculovirus*	2	A7		No
Flanders	FLAV	*Rhabdoviridae*	2	S	Hart Park	No

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Flexal	FLEV	*Arenavirus*	3	S	Tacaribe	No
Fomede	FV	*Orbivirus*	2	A7	Chobar Gorge	No
Forecariah	FORV	*Bunyaviridae*	2	A7	Bhanja	No
Fort Morgan	FMV	*Alphavirus*	2	S	A[f]	No
Fort Sherman	FSV	*Orthobunyavirus*	2	A7	Bunyamwera	No
Frijoles	FRIV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Gabek Forest	GFV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Gadgets Gully	GGYV	*Flavivirus*	2	IE	B[f]	No
Gamboa	GAMV	*Orthobunyavirus*	2	S	Gamboa	No
Gan Gan	GGV	*Bunyaviridae*	2	A7	Mapputta	No
Garba	GARV	*Rhabdoviridae*	2	IE	Matariva	No
Garissa	GRSV	*Orthobunyavirus*	3	A7	Bunyamwera	No
Germiston	GERV	*Orthobunyavirus*	3		Bunyamwera	Yes
Getah	GETV	*Alphavirus*	2	A1	A[f]	No
Gomoka	GOMV	*Orbivirus*	2	S	Ieri	No
Gordil	GORV	*Phlebovirus*	2	IE	Phlebotomus Fever	No
Gossas	GOSV	*Rhabdoviridae*	2	S		No
Grand Arbaud	GAV	*Phlebovirus*	2	S	Uukuniemi	No
Gray Lodge	GLOV	*Vesiculovirus*	2	IE	Vesicular Stomatitis	No
Great Island	GIV	*Orbivirus*	2	S	Kemerovo	No
Guajara	GJAV	*Orthobunyavirus*	2	S	Capim	No
Guama	GMAV	*Orthobunyavirus*	2	S	Guama	No
Guanarito	GTOV	*Arenavirus*	4	A4	Tacaribe	Yes
Guaratuba	GTBV	*Orthobunyavirus*	2	A7	Guama	No
Guaroa	GROV	*Orthobunyavirus*	2	S	California	No
Gumbo Limbo	GLV	*Orthobunyavirus*	2	S	C[f]	No
Gurupi	GURV	*Orbivirus*	2	IE	Changuinola	No
Hantaan	HTNV	*Hantavirus*	3[a]	S	Hantaan	No
Hanzalova	HANV	*Flavivirus*	4	A4	B[f]	Yes
Hart Park	HPV	*Rhabdoviridae*	2	S	Hart Park	No
Hazara	HAZV	*Nairovirus*	2	S	CHF-Congo	No
Highlands J	HJV	*Alphavirus*	2	S	A[f]	No
Huacho	HUAV	*Orbivirus*	2	S	Kemerovo	No

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Hughes	HUGV	*Nairovirus*	2	S	Hughes	No
Hypr	HYPRV	*Flavivirus*	4	S	B[f]	Yes
Iaco	IACOV	*Orthobunyavirus*	2	IE	Bunyamwera	No
Ibaraki	IBAV	*Orbivirus*	2	IE	Epizootic Hemorrhagic Disease	Yes
Icoaraci	ICOV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Ieri	IERIV	*Orbivirus*	2	S	Ieri	No
Ife	IFEV	*Orbivirus b*	2	IE		No
Iguape	IGUV	*Flavivirus*	2	A7	B[f]	No
Ilesha	ILEV	*Orthobunyavirus*	2	S	Bunyamwera	No
Ilheus	ILHV	*Flavivirus*	2	S	B[f]	No
Ingwavuma	INGV	*Orthobunyavirus*	2	S	Simbu	No
Inhangapi	INHV	*Rhabdoviridae*	2	IE		No
Inini	INIV	*Orthobunyavirus*	2	IE	Simbu	No
Inkoo	INKV	*Orthobunyavirus*	2	S	California	No
Ippy	IPPYV	*Arenavirus*	2	S	Tacaribe	No
Iriri	IRRV	*Rhabdoviridae*	2	A7		No
Irituia	IRIV	*Orbivirus*	2	S	Changuinola	No
Isfahan	ISFV	*Vesiculovirus*	2	S	Vesicular Stomatitis	No
Israel Turkey Meningitis	ITV	*Flavivirus*	2 with 3 practices	S	B[f]	No
Issyk-Kul	ISKV	*Bunyaviridae*	3	IE		No
Itacaiunas	ITCNV	*Rhabdoviridae*	2	A7		No
Itaituba	ITAV	*Phlebovirus*	2	IE	Phlebotomus Fever	No
Itaporanga	ITPV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Itaqui	ITQV	*Orthobunyavirus*	2	S	C[f]	No
Itimirim	ITIV	*Orthobunyavirus*	2	IE	Guama	No
Itupiranga	ITUV	*Orbivirus b*	2	IE		No
Ixcanal	IXCV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Jacareacanga	JACV	*Orbivirus*	2	IE	Corriparta	No
Jacunda	JCNV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Jamanxi	JAMV	*Orbivirus*	2	IE	Changuinola	No
Jamestown Canyon	JCV	*Orthobunyavirus*	2	S	California	No

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Japanaut	JAPV	*Orbivirus b*	2	S		No
Japanese Encephalitis	JEV	*Flavivirus*	3[c]	S	B[f]	No
Jari	JARIV	*Orbivirus*	2	IE	Changuinola	No
Jatobal	JTBV	*Orthobunyavirus*	2	A7		No
Jerry Slough	JSV	*Orthobunyavirus*	2	S	California	No
Joa	JOAV	*Phlebovirus*	2	A7		No
Johnston Atoll	JAV	Unassigned	2	S	Quaranfil	No
Joinjakaka	JOIV	*Rhabdoviridae*	2	S		No
Juan Diaz	JDV	*Orthobunyavirus*	2	S	Capim	No
Jugra	JUGV	*Flavivirus*	2	S	B[f]	No
Junin	JUNV	*Arenavirus*	4	A6	Tacaribe	Yes
Jurona	JURV	*Vesiculovirus*	2	S	Vesicular Stomatitis	No
Juruaca	JRCV	*Picornavirus* [b]	2	A7		No
Jutiapa	JUTV	*Flavivirus*	2	S	B[f]	No
Kadam	KADV	*Flavivirus*	2	S	B[f]	No
Kaeng Khoi	KKV	*Orthobunyavirus* [b]	2	S		No
Kaikalur	KAIV	*Orthobunyavirus*	2	S	Simbu	No
Kairi	KRIV	*Orthobunyavirus*	2	A1	Bunyamwera	No
Kaisodi	KSOV	*Bunyaviridae*	2	S	Kaisodi	No
Kamese	KAMV	*Rhabdoviridae*	2	S	Hart Park	No
Kamiti River	KRV	*Flavivirus*	2	A7		No
Kammavanpettai	KMPV	*Orbivirus*	2	S		No
Kannamangalam	KANV	*Rhabdoviridae*	2	S		No
Kao Shuan	KSV	*Nairovirus*	2	S	Dera Ghazi Khan	No
Karimabad	KARV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Karshi	KSIV	*Flavivirus*	2	S	B[f]	No
Kasba	KASV	*Orbivirus*	2	S	Palyam	No
Kedougou	KEDV	*Flavivirus*	2	A7	B[f]	No
Kemerovo	KEMV	*Orbivirus*	2	S	Kemerovo	No
Kern Canyon	KCV	*Rhabdoviridae*	2	S	Kern Canyon	No
Ketapang	KETV	*Orthobunyavirus*	2	S	Bakau	No
Keterah	KTRV	*Bunyaviridae*	2	S		No
Keuraliba	KEUV	*Rhabdoviridae*	2	S	Le Dantec	No

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Keystone	KEYV	*Orthobunyavirus*	2	S	California	No
Khabarovsk	KHAV	*Hantavirus*	3[a]	IE	Hantaan	No
Khasan	KHAV	*Nairovirus*	2	IE	CCHF	No
Kimberley	KIMV	*Lyssavirus*	2	A7	Bovine Ephemeral Fever	No
Kindia	KINV	*Orbivirus*	2	A7	Palyam	No
Kismayo	KISV	*Bunyaviridae*	2	S	Bhanja	No
Klamath	KLAV	*Vesiculovirus*	2	S	Vesicular Stomatitis	No
Kokobera	KOKV	*Flavivirus*	2	S	B[f]	No
Kolongo	KOLV	*Lyssavirus*	2	S	Rab	No
Koongol	KOOV	*Orthobunyavirus*	2	S	Koongol	No
Kotonkan	KOTV	*Lyssavirus*	2	S	Rab	No
Koutango	KOUV	*Flavivirus*	3	S	B[f]	No
Kowanyama	KOWV	*Bunyaviridae*	2	S		No
Kumlinge	KUMV	*Flavivirus*	4	A4	B[f]	Yes
Kunjin	KUNV	*Flavivirus*	2	S	B[f]	No
Kununurra	KNAV	*Rhabdoviridae*	2	S		No
Kwatta	KWAV	*Vesiculovirus*	2	S	Vesicular Stomatitis	No
Kyasanur Forest Disease	KFDV	*Flavivirus*	4	S	B[f]	Yes
Kyzylagach	KYZV	*Alphavirus*	2	IE	A[f]	No
La Crosse	LACV	*Orthobunyavirus*	2	S	California	No
Lagos Bat	LBV	*Lyssavirus*	2	S	Rab	No
Laguna Negra	LANV	*Hantavirus*	3[a]	IE		No
La Joya	LJV	*Vesiculovirus*	2	S	Vesicular Stomatitis	No
Lake Clarendon	LCV	*Orbivirus b*	2	IE		No
Landjia	LJAV	*Rhabdoviridae*	2	S		No
Langat	LGTV	*Flavivirus*	2	S	B[f]	No
Lanjan	LJNV	*Bunyaviridae*	2	S	Kaisodi	No
Las Maloyas	LMV	*Orthobunyavirus*	2	A7	Anopheles A	No
Lassa	LASV	*Arenavirus*	4	S	Tacaribe	Yes
Latino	LATV	*Arenavirus*	2	A5	Tacaribe	No
Lebombo	LEBV	*Orbivirus*	2	S		No
Lechiguanas	LECHV	*Hantavirus*	3[a]	IE	Hantaan	No

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Le Dantec	LDV	*Rhabdoviridae*	2	S	Le Dantec	No
Lednice	LEDV	*Orthobunyavirus*	2	A7	Turlock	No
Lipovnik	LIPV	*Orbivirus*	2	S	Kemerovo	No
Llano Seco	LLSV	*Orbivirus*	2	IE	Umatilla	No
Lokern	LOKV	*Orthobunyavirus*	2	S	Bunyamwera	No
Lone Star	LSV	*Bunyaviridae*	2	S		No
Louping Ill	LIV	*Flavivirus*	3[c]	S	B[f]	Yes
Lukuni	LUKV	*Orthobunyavirus*	2	S	Anopheles A	No
Macaua	MCAV	*Orthobunyavirus*	2	IE	Bunyamwera	No
Machupo	MACV	*Arenavirus*	4	S	Tacaribe	Yes
Madrid	MADV	*Orthobunyavirus*	2	S	C[f]	No
Maguari	MAGV	*Orthobunyavirus*	2	S	Bunyamwera	No
Mahogany Hammock	MHV	*Orthobunyavirus*	2	S	Guama	No
Main Drain	MDV	*Orthobunyavirus*	2	S	Bunyamwera	No
Malakal	MALV	*Lyssavirus*	2	S	Bovine Ephemeral	No
Manawa	MWAV	*Phlebovirus*	2	S	Uukumiemi	No
Manitoba	MNTBV	*Rhabdoviridae*	2	A7		No
Manzanilla	MANV	*Orthobunyavirus*	2	S	Simbu	No
Mapputta	MAPV	*Bunyaviridae*	2	S	Mapputta	No
Maporal	MPRLV	*Hantavirus*	3[a]	IE	Hantaan	No
Maprik	MPKV	*Bunyaviridae*	2	S	Mapputta	No
Maraba	MARAV	*Vesiculovirus*	2	A7		No
Marajo	MRJV	Unassigned	2	IE		No
Marburg	MARV	*Filovirus*	4	S	Marburg	Yes
Marco	MCOV	*Rhabdoviridae*	2	S		No
Mariquita	MRQV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Marituba	MTBV	*Orthobunyavirus*	2	S	C[f]	No
Marrakai	MARV	*Orbivirus*	2	S	Palyam	No
Matariya	MTYV	*Rhabdoviridae*	2	S	Matariva	No
Matruh	MTRV	*Orthobunyavirus*	2	S	Tete	No
Matucare	MATV	*Orbivirus*	2	S		No
Mayaro	MAYV	*Alphavirus*	2	S	A[f]	No
Mboke	MBOV	*Orthobunyavirus*	2	A7	Bunyamwera	No
Meaban	MEAV	*Flavivirus*	2	IE	B[f]	No

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Melao	MELV	*Orthobunyavirus*	2	S	California	No
Mermet	MERV	*Orthobunyavirus*	2	S	Simbu	No
Middelburg	MIDV	*Alphavirus*	2	A1	A[f]	No
Minatitlan	MNTV	*Orthobunyavirus*	2	S	Minatitlan	No
Minnal	MINV	*Orbivirus*	2	S	Umatilla	No
Mirim	MIRV	*Orthobunyavirus*	2	S	Guama	No
Mitchell River	MRV	*Orbivirus*	2	S		No
Mobala	MOBV	*Arenavirus*	3	A7	Tacaribe	No
Modoc	MODV	*Flavivirus*	2	S	B[f]	No
Moju	MOJUV	*Orthobunyavirus*	2	S	Guama	No
Mojui Dos Campos	MDCV	*Orthobunyavirus*	2	IE		No
Mono Lake	MLV	*Orbivirus*	2	S	Kemerovo	No
Mont. Myotis Leukemia	MMLV	*Flavivirus*	2	S	B[f]	No
Monte Dourado	MDOV	*Orbivirus*	2	IE	Changuinola	No
Mopeia	MOPV	*Arenavirus*	3	A7		No
Moriche	MORV	*Orthobunyavirus*	2	S	Capim	No
Morro Bay	MBV	*Orthobunyavrius*	2	IE	California	No
Morumbi	MRMBV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Mosqueiro	MQOV	*Rhabdoviridae*	2	A7	Hart Park	No
Mossuril	MOSV	*Rhabdoviridae*	2	S	Hart Park	No
Mount Elgon Bat	MEBV	*Vesiculovirus*	2	S	Vesicular Stomatitis	No
M'Poko	MPOV	*Orthobunyavirus*	2	S	Turlock	No
Mucambo	MUCV	*Alphavirus*	3	S	A[f]	Yes
Mucura	MCRV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Munguba	MUNV	*Phlebovirus*	2	IE	Phlebotomus Fever	No
Murray Valley Encephalitis	MVEV	*Flavivirus*	3	S	B[f]	No
Murutucu	MURV	*Orthobunyavirus*	2	S	C[f]	No
Mykines	MYKV	*Orbivirus*	2	A7	Kemerovo	No
Nairobi Sheep Disease	NSDV	*Nairovirus*	3[c]	A1	Nairobi Sheep Disease	No
Naranjal	NJLV	*Flavivirus*	2	IE	B[f]	No
Nariva	NARV	*Paramyxoviridae*	2	IE		No
Nasoule	NASV	*Lyssavirus*	2	A7	Rab	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Navarro	NAVV	*Rhabdoviridae*	2	S		No
Ndelle	NDEV	*Orthoreovirus*	2	A7	Ndelle	No
Ndumu	NDUV	*Alphavirus*	2	A1	A[f]	No
Negishi	NEGV	*Flavivirus*	3	S	B[f]	No
Nepuyo	NEPV	*Orthobunyavirus*	2	S	C[f]	No
Netivot	NETV	*Orbivirus*	2	A7		No
New Minto	NMV	*Rhabdoviridae*	2	IE	Sawgrass	No
Ngaingan	NGAV	*Lyssavirus*	2	S	Tibrogargan	No
Ngari d	NRIV	*Orthobunyavirus*	3	A7	Bunyamera	No
Ngoupe	NGOV	*Orbivirus*	2	A7	Eubenangee	No
Nique	NIQV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Nkolbisson	NKOV	*Rhabdoviridae*	2	S	Kern Canyon	No
Nodamura	NOV	*Alphanodavirus*	2	IE		No
Nola	NOLAV	*Orthobunyavirus*	2	S	Bakau	No
Northway	NORV	*Orthobunyavirus*	2	IE	Bunyamwera	No
Ntaya	NTAV	*Flavivirus*	2	S	B[f]	No
Nugget	NUGV	*Orbivirus*	2	S	Kemerovo	No
Nyamanini	NYMV	Unassigned	2	S	Nyamanini	No
Nyando	NDV	*Orthobunyavirus*	2	S	Nyando	No
Oak Vale	OVV	*Rhabdoviridae*	2	A7		No
Odrenisrou	ODRV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Okhotskiy	OKHV	*Orbivirus*	2	S	Kemerovo	No
Okola	OKOV	*Bunyaviridae*	2	S	Tanga	No
Olifantsvlei	OLIV	*Orthobunyavirus*	2	S	Olifantsylei	No
Omo	OMOV	*Nairovirus*	2	A7	Qalyub	No
Omsk Hemorrhagic	OHFV	*Flavivirus*	4	S	B[f]	Yes
O'Nyong-Nyong	ONNV	*Alphavirus*	2	S	A[f]	Yes
Oran	ORANV	*Hantavirus*	3[a]	IE	Hantaan	No
Oriboca	ORIV	*Orthobunyavirus*	2	S	C[f]	No
Oriximina	ORXV	*Phlebovirus*	2	IE	Phlebotomus Fever	No
Oropouche	OROV	*Orthobunyavirus*	3	S	Simbu	Yes
Orungo	ORUV	*Orbivirus*	2	S	Orungo	No
Ossa	OSSAV	*Orthobunyavirus*	2	S	C[f]	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Ouango	OUAV	*Rhabdoviridae*	2	IE		No
Oubangui	OUBV	*Poxviridae*	2	IE		No
Oubi	OUBIV	*Orthobunyavirus*	2	A7	Olifantsylei	No
Ourem	OURV	*Orbivirus*	2	IE	Changuinola	No
Pacora	PCAV	*Bunyaviridae*	2	S		No
Pacui	PACV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Pahayokee	PAHV	*Orthobunyavirus*	2	S	Patois	No
Palma	PMAV	*Bunyaviridae*	2	IE	Bhanja	No
Palestina	PLSV	*Orthobunyavirus*	2	IE	Minatitlan	No
Palyam	PALV	*Orbivirus*	2	S	Palyam	No
Para	PARAV	*Orthobunyavirus*	2	IE	Simbu	No
Paramushir	PMRV	*Nairovirus*	2	IE	Sakhalin	No
Parana	PARV	*Arenavirus*	2	A5	Tacaribe	No
Paroo River	PRV	*Orbivirus*	2	IE		No
Pata	PATAV	*Orbivirus*	2	S		No
Pathum Thani	PTHV	*Nairovirus*	2	S	Dera Ghazi Khan	No
Patois	PATV	*Orthobunyavirus*	2	S	Patois	No
Peaton	PEAV	*Orthobunyavirus*	2	A1	Simbu	No
Pergamino	PRGV	*Hantavirus*	3[a]	IE		No
Perinet	PERV	*Vesiculovirus*	2	A7	Vesicular Stomatitis	No
Petevo	PETV	*Orbivirus*	2	A7	Palyam	No
Phnom-Penh Bat	PPBV	*Flavivirus*	2	S	Bf	No
Pichinde	PICV	*Arenavirus*	2	A5	Tacaribe	No
Picola	PIAV	*Orbivirus*	2	IE	Wongorr	No
Pirital	PIRV	*Arenavirus*	3	IE		No
Piry	PIRYV	*Vesiculovirus*	3	S	Vesicular Stomatitis	No
Pixuna	PIXV	*Alphavirus*	2	S	A[f]	No
Playas	PLAV	*Orthobunyavirus*	2	IE	Bunyamwera	No
Pongola	PGAV	*Orthobunyavirus*	2	S	Bwamba	No
Ponteves	PTVV	*Phlebovirus*	2	A7	Uukuniemi	No
Potosi	POTV	*Orthobunyavirus*	2	IE	Bunyamwera	No
Powassan	POWV	*Flavivirus*	3	S	B[f]	No
Precarious Point	PPV	*Phlebovirus*	2	A7	Uukuniemi	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Pretoria	PREV	*Nairovirus*	2	S	Dera Ghazi Khan	No
Prospect Hill	PHV	*Hantavirus*	2	A8	Hantaan	No
Puchong	PUCV	*Lyssavirus*	2	S	Bovine Ephemeral ever	No
Pueblo Viejo	PVV	*Orthobunyavirus*	2	IE	Gamboa	No
Punta Salinas	PSV	*Nairovirus*	2	S	Hughes	No
Punta Toro	PTV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Purus	PURV	*Orbivirus*	2	IE	Changuinola	No
Puumala	PUUV	*Hantavirus*	3[a]	IE	Hantaan	No
Qalyub	QYBV	*Nairovirus*	2	S	Qalyub	No
Quaranfil	QRFV	Unassigned	2	S	Quaranfil	No
Radi	RADIV	*Vesiculovirus*	2	A7	Vesicular Stomatitis	No
Razdan	RAZV	*Bunyaviridae*	2	IE		No
Resistencia	RTAV	*Bunyaviridae*	2	IE	Resistencia	No
Restan	RESV	*Orthobunyavirus*	2	S	C[f]	No
Rhode Island	RHIV	*Rhabdoviridae*	2	A7		No
Rift Valley Fever	RVFV	*Phlebovirus*	3[c]	S	Phlebotomus Fever	Yes
Rio Bravo	RBV	*Flavivirus*	2	S	B[f]	No
Rio Grande	RGV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Rio Preto	RIOPV	Unassigned	2	IE		No
Rochambeau	RBUV	*Lyssavirus*	2	IE	Rab	No
Rocio	ROCV	*Flavivirus*	3	S	B[f]	Yes
Ross River	RRV	*Alphavirus*	2	S	A[f]	No
Royal Farm	RFV	*Flavivirus*	2	S	B[f]	No
Russian Spring-Summer Encephalitis	RSSEV	*Flavivirus*	4	S	B[f]	Yes
Saaremaa	SAAV	*Hantavirus*	3[a]	IE	Hantaan	No
Sabia	SABV	*Arenavirus*	4	A4		Yes
Sabo	SABOV	*Orthobunyavirus*	2	S	Simbu	No
Saboya	SABV	*Flavivirus*	2	S	B[f]	No
Sagiyama	SAGV	*Alphavirus*	2	A1	A[f]	No
Saint-Floris	SAFV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Sakhalin	SAKV	*Nairovirus*	2	S	Sakhalin	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Salanga	SGAV	*Poxviridae*	2	IE	SGA	No
Salehabad	SALV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Salmon River	SAVV	*Coltivirus*	2	IE	Colorado Tick Fever	No
Sal Vieja	SVV	*Flavivirus*	2	A7	B[f]	No
San Angelo	SAV	*Orthobunyavirus*	2	S	California	No
Sandfly Fever, Naples	SFNV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Sandfly Fever, Sicilian	SFSV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Sandjimba	SJAV	*Lyssavirus*	2	S	Rab	No
Sango	SANV	*Orthobunyavirus*	2	S	Simbu	No
San Juan	SJV	*Orthobunyavirus*	2	IE	Gamboa	No
San Perlita	SPV	*Flavivirus*	2	A7	B[f]	No
Santarem	STMV	*Bunyaviridae*	2	IE		No
Santa Rosa	SARV	*Orthobunyavirus*	2	IE	Bunyamwera	No
Saraca	SRAV	*Orbivirus*	2	IE	Changuinola	No
Sathuperi	SATV	*Orthobunyavirus*	2	S	Simbu	No
Saumarez Reef	SREV	*Flavivirus*	2	IE	B[f]	No
Sawgrass	SAWV	*Rhabdoviridae*	2	S	Sawgrass	No
Sebokele	SEBV	Unassigned	2	S		No
Sedlec	SEDV	*Bunyaviridae*	2	A7		No
Seletar	SELV	*Orbivirus*	2	S	Kemerovo	No
Sembalam	SEMV	Unassigned	2	S		No
Semliki Forest	SFV	*Alphavirus*	3	A2	A[f]	No
Sena Madureira	SMV	*Rhabdoviridae*	2	IE	Timbo	No
Seoul	SEOV	*Hantavirus*	3[a]	IE	Hantaan	No
Sepik	SEPV	*Flavivirus*	2	IE	B[f]	No
Serra Do Navio	SDNV	*Orthobunyavirus*	2	A7	California	No
Serra Norte	SRNV	*Phlebovirus*	2	A7		No
Shamonda	SHAV	*Orthobunyavirus*	2	S	Simbu	No
Shark River	SRV	*Orthobunyavirus*	2	S	Patois	No
Shokwe	SHOV	*Orthobunyavirus*	2	IE	Bunyamwera	No
Shuni	SHUV	*Orthobunyavirus*	2	S	Simbu	No
Silverwater	SILV	*Bunyaviridae*	2	S	Kaisodi	No
Simbu	SIMV	*Orthobunyavirus*	2	S	Simbu	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Simian Hemorrhagic Fever	SHFV	*Arterivirus*	2	A2	Simian Hemorrhagic Fever	No
Sindbis	SINV	*Alphavirus*	2	S	A[f]	No
Sin Nombre	SNV	*Hantavirus*	3[a]	IE	Hantaan	No
Sixgun City	SCV	*Orbivirus*	2	S	Kemerovo	No
Slovakia	SLOV	Unassigned	3	IE		No
Snowshoe Hare	SSHV	*Orthobunyavirus*	2	S	California	No
Sokoluk	SOKV	*Flavivirus*	2	S	B[f]	No
Soldado	SOLV	*Nairovirus*	2	S	Hughes	No
Somone	SOMV	Unassigned	3	IE	Somone	No
Sororoca	SORV	*Orthobunyavirus*	2	S	Bunyamwera	No
Spondweni	SPOV	*Flavivirus*	2	S	B[f]	No
Sripur	SRIV	*Rhabdoviridae*	3	IE		No
St. Louis Encephalitis	SLEV	*Flavivirus*	3	S	B[f]	No
Stratford	STRV	*Flavivirus*	2	S	B[f]	No
Sunday Canyon	SCAV	*Bunyaviridae*	2	S		No
Tacaiuma	TCMV	*Orthobunyavirus*	2	S	Anopheles A	No
Tacaribe	TCRV	*Arenavirus*	2	A5	Tacaribe	No
Taggert	TAGV	*Nairovirus*	2	S	Sakhalin	No
Tahyna	TAHV	*Orthobunyavirus*	2	S	California	No
Tai	TAIV	*Bunyaviridae*	2	A7	Bunyamwera	No
Tamdy	TDYV	*Bunyaviridae*	2	IE		No
Tamiami	TAMV	*Arenavirus*	2	A5	Tacaribe	No
Tanga	TANV	*Bunyaviridae*	2	S	Tanga	No
Tanjong Rabok	TRV	*Orthobunyavirus*	2	S	Bakau	No
Tapara	TAPV	*Phlebovirus*	2	A7		No
Tataguine	TATV	*Bunyaviridae*	2	S		No
Tehran	THEV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Telok Forest	TFV	*Orthobunyavirus*	2	IE	Bakau	No
Tembe	TMEV	*Orbivirus b*	2	S		No
Tembusu	TMUV	*Flavivirus*	2	S	B[f]	No
Tensaw	TENV	*Orthobunyavirus*	2	S	Bunyamwera	No
Termeil	TERV	*Bunyavirus b*	2	IE		No
Tete	TETEV	*Orthobunyavirus*	2	S	Tete	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Thiafora	TFAV	*Bunyaviridae*	2	A7	Thiafora	No
Thimiri	THIV	*Orthobunyavirus*	2	S	Simbu	No
Thogoto	THOV	*Orthomyxoviridae*	2	S	Thogoto	No
Thottapalayam	TPMV	*Hantavirus*	2	S	Hantaan	No
Tibrogargan	TIBV	*Lyssavirus*	2	S	Tibrogargan	No
Tilligerry	TILV	*Orbivirus*	2	IE	Eubenangee	No
Timbo	TIMV	*Rhabdoviridae*	2	S	Timbo	No
Timboteua	TBTV	*Orthobunyavirus*	2	A7	Guama	No
Tinaroo	TINV	*Orthobunyavirus*	2	IE	Simbu	No
Tindholmur	TDMV	*Orbivirus*	2	A7	Kemerovo	No
Tlacotalpan	TLAV	*Orthobunyavirus*	2	IE	Bunyamwera	No
Tonate	TONV	*Alphavirus*	3	IE	A[f]	Yes
Topografov	TOPV	*Hantavirus*	3[a]	IE	Hantaan	No
Toscana	TOSV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Toure	TOUV	Unassigned	2	S		No
Tracambe	TRCV	*Orbivirus*	2	A7		No
Tribec	TRBV	*Orbivirus*	2	S	Kemerovo	No
Triniti	TNTV	*Togaviridae*	2	S		No
Trivittatus	TVTV	*Orthobunyavirus*	2	S	California	No
Trocara	TROCV	*Alphavirus*	2	IE	A[f]	No
Trombetas	TRMV	*Orthobunyavirus*	2	A7		No
Trubanaman	TRUV	*Bunyaviridae*	2	S	Mapputta	No
Tsuruse	TSUV	*Orthobunyavirus*	2	S	Tete	No
Tucurui	TUCRV	*Orthobunyavirus*	2	A7		No
Tula	TULV	*Hantavirus*	2	A8		No
Tunis	TUNV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Turlock	TURV	*Orthobunyavirus*	2	S	Turlock	No
Turuna	TUAV	*Phlebovirus*	2	IE	Phlebotomus Fever	No
Tyuleniy	TYUV	*Flavivirus*	2	S	B[f]	No
Uganda S	UGSV	*Flavivirus*	2	S	B[f]	No
Umatilla	UMAV	*Orbivirus*	2	S	Umatilla	No
Umbre	UMBV	*Orthobunyavirus*	2	S	Turlock	No
Una	UNAV	*Alphavirus*	2	S	A[f]	No
Upolu	UPOV	*Bunyaviridae*	2	S	Upolu	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Uriurana	UURV	*Phlebovirus*	2	A7	Phlebotomus Fever	No
Urucuri	URUV	*Phlebovirus*	2	S	Phlebotomus Fever	No
Usutu	USUV	*Flavivirus*	2	S	B[f]	No
Utinga	UTIV	*Orthobunyavirus*	2	IE	Simbu	No
Uukuniemi	UUKV	*Phlebovirus*	2	S	Uukuniemi	No
Vellore	VELV	*Orbivirus*	2	S	Palyam	No
Venezuelan Equine Encephalitis	VEEV	*Alphavirus*	3[c]	S	A[f]	Yes
Venkatapuram	VKTV	Unassigned	2	S		No
Vinces	VINV	*Orthobunyavirus*	2	A7	C[f]	No
Virgin River	VRV	*Orthobunyavirus*	2	A7	Anopheles A	No
Vesicular Stomatitis-Alagoas	VSAV	*Vesiculovirus*	2[c]	S	Vesicular Stomatitis	No
Vesicular Stomatitis-Indiana	VSIV	*Vesiculovirus*	2[c]	A3	Vesicular Stomatitis	No
Vesicular Stomatitis-New Jersey	VSNJV	*Vesiculovirus*	2[c]	A3	Vesicular Stomatitis	No
Wad Medani	WMV	*Orbivirus*	2	S	Kemerovo	No
Wallal	WALV	*Orbivirus*	2	S	Wallal	No
Wanowrie	WANV	*Bunyaviridae*	2	S		No
Warrego	WARV	*Orbivirus*	2	S	Warrego	No
Wesselsbron	WESSV	*Flavivirus*	3[c]	S	B[f]	Yes
Western Equine Encephalitis	WEEV	*Alphavirus*	3	S	A[f]	No
West Nile	WNV	*Flavivirus*	3	S	B[f]	No
Whataroa	WHAV	*Alphavirus*	2	S	A[f]	No
Whitewater Arroyo	WWAV	*Arenavirus*	3	IE	Tacaribe	No
Witwatersrand	WITV	*Bunyaviridae*	2	S		No
Wongal	WONV	*Orthobunyavirus*	2	S	Koongol	No
Wongorr	WGRV	*Orbivirus*	2	S	Wongorr	No
Wyeomyia	WYOV	*Orthobunyavirus*	2	S	Bunyamwera	No
Xiburema	XIBV	*Rhabdoviridae*	2	IE		No
Xingu	XINV	*Orthobunyavirus*	3			No
Yacaaba	YACV	*Bunyaviridae*	2	IE		No
Yaounde	YAOV	*Flavivirus*	2	A7	B[f]	No

Name	Acronym	Taxonomic Status (Family or Genus)	Recommended Biosafety Level	Basis of Rating	Antigenic Group	HEPA Filtration on Lab Exhaust
Yaquina Head	YHV	*Orbivirus*	2	S	Kemerovo	No
Yata	YATAV	*Rhabdoviridae*	2	S		No
Yellow Fever	YFV	*Flavivirus*	3	S	B[f]	Yes
Yogue	YOGV	*Bunyaviridae*	2	S	Yogue	No
Yoka	YOKA	*Poxviridae*	2	IE		No
Yug Bogdanovac	YBV	*Vesiculovirus*	2	IE	Vesicular Stomatitis	No
Zaliv Terpeniya	ZTV	*Phlebovirus*	2	S	Uukuniemi	No
Zegla	ZEGV	*Orthobunyavirus*	2	S	Patois	No
Zika	ZIKV	*Flavivirus*	2	S	B[f]	No
Zirqa	ZIRV	*Nairovirus*	2	S	Hughes	No

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

[a] Containment requirements will vary based on virus concentration, animal species, or virus type. See the Hantavirus agent summary statement in the viral agent chapter.

[b] Tentative placement in the genus.

[c] These organisms are considered pathogens of significant agricultural importance by the USDA (see Appendix D) and may require additional containment (up to and including BSL-3-Ag 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.

[d] Alternate name for Ganjam virus.

[e] Garissa virus is considered an isolate of this virus, so same containment requirements apply.

[f] Antigenic groups designated A, B, and C refer to the original comprehensive and unifying serogroups established by Casals, Brown, and Whitman based on cross-reactivity among known arboviruses (2,21). Group A viruses are members of the genus *Alphavirus*, group B belong to the family *Flaviviridae*, and Group C viruses are members of the family *Bunyaviridae.*

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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). Each BoNT holotoxin is a disulfide-bonded heterodimer composed of a zinc metallo-protease "light chain" (approximately 50 kD) and a receptor binding "heavy chain" (approximately 100 kD). The heavy chain enhances cell binding and translocation of the catalytic light chain across the vesicular membrane.[1] There are also a number of important accessory proteins that can stabilize the natural toxin complex in biological systems or in buffer.

Four of the serotypes (A, B, E and, less commonly, F) are responsible for most human poisoning through contaminated food, wound infection, or infant botulism, whereas livestock may be at greater risk for poisoning with serotypes B, C1 and D.[2,3] It is important to recognize, however, that all BoNT serotypes are highly toxic and lethal by injection or aerosol delivery. BoNT is one of the most toxic proteins known; absorption of less than one microgram (µg) of BoNT can cause severe incapacitation or death, depending upon the serotype and the route of exposure.

Diagnosis of Laboratory Exposures

Botulism is primarily clinically diagnosed through physician observations of signs and symptoms that are similar for all serotypes and all routes of intoxication.[4] There typically is a latency of several hours to days, depending upon the amount of toxin absorbed, before the signs and symptoms of BoNT poisoning occur. The first symptoms of exposure generally include blurred vision, dry mouth and difficulty swallowing and speaking. This is followed by a descending, symmetrical flaccid paralysis, which can progress to generalized muscle weakness and respiratory failure. Sophisticated tests such as nerve conduction studies and single-fiber electromyography can support the diagnosis and distinguish it from similar neuromuscular conditions. Routine laboratory tests are of limited value because of the low levels of BoNT required to intoxicate, as well as the delay in onset of symptoms.

Laboratory Safety and Containment Recommendations

Solutions of sodium hypochlorite (0.1%) or sodium hydroxide (0.1N) readily inactivate the toxin and are recommended for decontamination of work surfaces and for spills. Additional considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Because neurotoxin producing Clostridia species requires an anaerobic environment for growth and it is 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 or growing neurotoxin producing Clostridia species cultures are unintentional aerosol generation, especially during centrifugation, and accidental needle-stick. Although BoNT does not penetrate intact skin,

proteins can be absorbed through broken or lacerated skin and, therefore, BoNT samples or contaminated material should be handled with gloves.

Workers in diagnostic laboratories should be aware that neurotoxin producing Clostridia species or its spores can be stable for weeks or longer in a variety of food products, clinical samples (e.g., serum, 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, but useful comparative data are available from the food industry. 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.[5]

A documented incident of laboratory intoxication with BoNT occurred in workers who were performing necropsies on animals that had been exposed 24 h earlier to aerosolized BoNT serotype A; the laboratory workers presumably inhaled aerosols generated from the animal fur. The intoxications were relatively mild, and all affected individuals recovered after a week of hospitalization.[6] Despite the low incidence of laboratory-associated botulism, the remarkable toxicity of BoNT necessitates that laboratory workers exercise caution during all experimental procedures.

BSL-2 practices, containment equipment, and facilities are recommended for routine dilutions, titrations or diagnostic studies with materials known to contain or have the potential to contain BoNT. Additional primary containment and personnel precautions, such as those recommended for BSL-3, should be implemented for activities with a high potential for aerosol or droplet production, or for those requiring routine handling of larger quantities of toxin.

Personnel not directly involved in laboratory studies involving botulinum toxin, such as maintenance personnel, should be discouraged from entering the laboratory when BoNT is in use until after the toxin and all work surfaces have been decontaminated. Purified preparations of toxin components, e.g. isolated BoNT “light chains” or “heavy chains,” should be handled as if contaminated with holotoxin unless proven otherwise by toxicity bioassays.

Special Issues

Vaccines A pentavalent (A, B, C, D and E) botulinum toxoid vaccine (PBT) is available through the CDC as an IND. Vaccination is recommended for all personnel working in direct contact with cultures of neurotoxin producing Clostridia species or stock solutions of BoNT. Due to a possible decline in the immunogenicity of available PBT stocks for some toxin serotypes, the immunization schedule for the PBT recently has been modified to require injections at 0, 2, 12, and 24 weeks, followed by a booster at 12 months and annual boosters thereafter. Since there is a possible decline in vaccine efficacy, the current vaccine contains toxoid for only 5 of the 7 toxin types, this vaccine should not be considered as the sole means of protection and should not replace other worker protection measures.

Select Agent Botulinum toxin is a select agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer if quantities are above the minimum exemption level. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA 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.

Staphylococcal Enterotoxins (SE)

SE are a group of closely related extracellular protein toxins of 23 to 29 kD molecular weight that are produced by distinct gene clusters found in a wide variety of *S. aureus* strains.[8,9] 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 man through pathological amplification of the normal T-cell receptor response, cytokine/lymphokine release, immunosuppression and endotoxic shock.[9,10]

SE serotype A (SEA) is a common cause of severe gastroenteritis in humans.[11] It has been estimated from accidental food poisoning that exposure to as little as 0.05 to 1 µg SEA by the gastric route causes incapacitating illness.[12-15] Comparative human toxicity for different serotypes of SE is largely unknown, but human volunteers exposed to 20-25 µg SE serotype B (SEB) in distilled water experienced enteritis similar to that caused by SEA.[16]

SE are highly toxic by intravenous and inhalation routes of exposure. By inference from accidental exposure of laboratory workers and controlled experiments with NHP, it has been estimated that inhalation of less than 1 ng/kg SEB can incapacitate more than 50% of exposed humans, and that the inhalation LD_{50} in humans may be as low as 20 ng/kg SEB.[17]

Exposure of mucous membranes to SE in a laboratory setting has been reported to cause incapacitating gastrointestinal symptoms, conjunctivitis and localized cutaneous swelling.[18]

Diagnosis of Laboratory Exposures

Diagnosis of SE intoxication is based on clinical and epidemiologic features. Gastric intoxication with SE begins rapidly after exposure (1-4 h) and is characterized by severe vomiting, sometimes accompanied by diarrhea, but without a high fever. At higher exposure levels, intoxication progresses to hypovolemia, dehydration, vasodilatation in the kidneys, and lethal shock.[11]

While fever is uncommon after oral ingestion, inhalation of SE causes a marked fever and respiratory distress. Inhalation of SEB causes a severe, incapacitating illness of rapid onset (3-4 h) lasting 3 to 4 days characterized by high fever, headache, and a nonproductive cough; swallowing small amounts of SE during an inhalation exposure may result in gastric symptoms as well.[19]

Differential diagnosis of SE inhalation may be unclear initially because the symptoms are similar to those caused by several respiratory pathogens such as influenza, adenovirus, and mycoplasma. Naturally occurring pneumonias or influenza, however, would typically involve patients presenting over a more prolonged interval of time, whereas SE intoxication tends to plateau rapidly, within a few hours. Nonspecific laboratory findings of SE inhalation include a neutrophilic leukocytosis, an elevated erythrocyte sedimentation rate, and chest X-ray abnormalities consistent with pulmonary edema.[19]

Laboratory confirmation of intoxication includes SE detection by immunoassay of environmental and clinical samples, and gene amplification to detect staphylococcal genes in environmental samples. 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. Respiratory secretions and nasal swabs may demonstrate the toxin early (within 24 h of inhalation exposure). Evaluation of neutralizing antibody titers in acute and convalescent sera of exposed individuals can be undertaken, but may yield false positives resulting from pre-existing antibodies produced in response to natural SE exposure.

Laboratory Safety and Containment Recommendations

General considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Accidental ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes are believed to be the primary hazards of SE for laboratory and animal-care personnel. SE are relatively stable, monomeric proteins, readily soluble in water, and resistant to proteolytic degradation and temperature fluctuations. 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, or tissues of exposed animals. Additional care should be taken during necropsy of exposed animals or in handling clinical stool samples because SE toxins retain toxic activity throughout the digestive tract.

Accidental laboratory exposures to SE serotype B have been reviewed.[18] Documented accidents included inhalation of SE aerosols generated from pressurized equipment failure, as well as re-aerosolization of residual toxin from the fur of exposed animals. The most common cause of laboratory intoxication

with SE is expected to result from accidental self-exposure via the mucous membranes by touching contaminated hands to the face or eyes.

BSL-2 practices and 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 and careful hand-washing and laboratory decontamination should be strictly enforced when working with SE. Depending upon a risk assessment of the laboratory operation, the use of a disposable face mask may be required to avoid accidental ingestion.

BSL-3 facilities, equipment, and practices are indicated 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 Agent SE is a select agent 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 this agent may require CDC and/or USDA 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.

Ricin Toxin

Ricin is produced in maturing seeds of the castor bean, *Ricinus communis,* which has been recognized for centuries as a highly poisonous plant for humans and livestock.[20] Ricin belongs to a family of ribosome inactivating proteins from plants, including abrin, modeccin, and viscumin, that share a similar overall structure and mechanism of action.[21] 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 and a potent inhibitor of protein synthesis, whereas the B-chain is a relatively non-toxic lectin that facilitates toxin binding and internalization to target cells.[20]

Ricin is much less toxic by weight than is BoNT or SE, and published case reports suggest that intramuscular or gastric ingestion of ricin is rarely fatal in adults.[22] 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, for example, the LD_{50} by intravenous injection is about 5 µg/kg, whereas it is 20 mg/kg by intragasteric route.[23,24] The ricin aerosol LD_{50} for NHP is estimated to be 10-15 µg/kg.[17] The human lethal dose has not been established rigorously, but may be as low as 1-5 mg of ricin by injection 25 or by the aerosol route (extropolation from two species of NGP).

Diagnosis of Laboratory Exposures

The primary diagnosis is through clinical manifestations that vary greatly depending upon the route of exposure. Following inhalation exposure of NHP, there is typically a latency period of 24-72 h that may be characterized by loss of appetite and listlessness. The latency period progresses rapidly to severe pulmonary distress, depending upon the exposure level. Most of the pathology occurs in the lung and upper respiratory tract, including inflammation, bloody sputum, and pulmonary edema. Toxicity from ricin inhalation would be expected to progress despite treatment with antibiotics, as opposed to an infectious process. There would be no mediastinitis as seen with inhalation anthrax. Ricin patients would not be expected to 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 h), but generally require exposure to much higher levels of toxin compared with the inhalation route. Following intramuscular injection of ricin, symptoms may persist for days 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.[25]

Specific immunoassay of serum and respiratory secretions or immunohistochemical stains of tissue may be used where available to confirm a diagnosis. Ricin is an extremely immunogenic toxin, and paired acute and convalescent sera should be obtained from survivors for measurement of antibody response. Polymerase chain reaction (PCR) can detect residual castor bean DNA in most ricin preparations. Additional supportive clinical or diagnostic features, after aerosol exposure to ricin, may include the following: bilateral infiltrates on chest radiographs, arterial hypoxemia, neutrophilic leukocytosis, and a bronchial aspirate rich in protein.[24]

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.[26] However, purified preparations of natural ricin A-chain or B-chain, as well as crude extracts from castor beans, should be handled as if contaminated by ricin until proven otherwise by bioassay.

BSL-2 practices, containment equipment and facilities are recommended, especially a laboratory coat, gloves, and respiratory protection, when handling ricin toxin or potentially contaminated materials.

Ricin is a relatively non-specific cytotoxin and irritant that should be handled in the laboratory as a non-volatile toxic chemical. A BSC (Class II, Type B1 or B2) or a chemical fume hood equipped with an exhaust HEPA filter and charcoal filter are indicated for activities with a high potential for aerosol, such as powder samples, and the use of large quantities of toxin. Laboratory coat, gloves, and full-face respirator should be worn if there is a potential for creating a toxin aerosol.

Special Issues

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

Select Agent Ricin toxin is a select agent 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 this agent may require CDC and/or USDA 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.

Selected Low Molecular Weight (LMW) Toxins

LMW toxins comprise a structurally and functionally diverse class of natural poisons, ranging in size from several hundred to a few thousand daltons, that includes complex organic structures, as well as 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 their filtration and biodistribution properties, are

generally more stable and persistent in the environment, and may exhibit poor water-solubility necessitating the use of organic solvent; 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;[27,28] saxitoxin and related paralytic shellfish poisons produced by dinoflagellates of the *Gonyaulax* family;[29] tetrodotoxin from a number of marine animals,[30] brevetoxin from the dinoflagellate *Ptychodiscus brevis*;[31] palytoxin from marine coelenterates belonging to the genus *Palythoa*,[32] polypeptide conotoxins α-GI (includes GIA) and α-MI from the *Conus* genus of gastropod mollusks;[33] and the monocyclic polypeptide, microcystin-LR from freshwater cyanobacteria *Microcystis aeruginosa*.[34]

Trichothecene mycotoxins comprise a broad class of structurally complex, non-volatile sesquiterpene compounds that are potent inhibitors of protein synthesis.[27,28] 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.[27] T-2 is a lipid-soluble molecule that can be absorbed into the body rapidly through exposed mucosal surfaces.[35] 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.[27] 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.[17,27] 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.[27,35]

Saxitoxin and tetrodotoxin are paralytic marine toxins that interfere with normal function of the sodium channel in excitable cells of heart, muscle and neuronal tissue.[36] Animals exposed to 1-10 µg/kg toxin by parenteral routes typically develop a rapid onset of excitability, muscle spasm, and respiratory distress; death may occur within 10-15 minutes from respiratory paralysis.[29,37] Humans ingesting seafood contaminated with saxitoxin or tetrodotoxin show similar signs of toxicity, typically preceded by paresthesias of the lips, face and extremities.[36,38]

Brevetoxins are cyclic-polyether, paralytic shellfish neurotoxins produced by marine dinoflagellates that accumulate in filter-feeding mollusks and may cause human intoxication from ingestion of contaminated seafood, or by irritation from sea spray containing the toxin.[36] The toxin depolarizes and opens voltage-gated sodium ion channels, effectively making the sodium channel of affected nerve or muscle cells hyper-excitable. Symptoms of human ingestion are expected to

include paresthesias of the face, throat and fingers or toes, followed by dizziness, chills, muscle pains, nausea, gastroenteritis, and reduced heart rate. Brevetoxin has a parenteral LD_{50} of 200 µg/kg in mice and guinea pigs.[31] Guinea pigs exposed to a slow infusion of brevetoxin develop fatal respiratory failure within 30 minutes of exposure to 20 µg/kg toxin.[37]

Palytoxin is a structurally complex, articulated fatty acid associated with soft coral *Palythoa vestitus* that is capable of binding and converting the essential cellular Na+/K+ pump into a non-selective cation channel.[32,39] Palytoxin is among the most potent coronary vasoconstrictors known, killing animals within minutes by cutting off oxygen to the myocardium.[40] The LD_{50} for intravenous administration ranges from 0.025 to 0.45 µg/kg in different species of laboratory animals.[40] 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.[40] Palytoxin disrupts normal corneal function and causes irreversible blindness at topically applied levels of approximately 400 ng/kg, despite extensive rinsing after ocular instillation.[40]

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.[33] 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, however, 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.[33,41] The α-conotoxins act as potent nicotinic antagonists and the µ-conotoxins block the sodium channel.[33] Symptoms of envenomation depend upon the *Conus* species involved, generally occur rapidly after exposure (minutes), and range from severe pain to spreading numbness.[42] Severe intoxication results in muscle paralysis, blurred or double vision, difficulty breathing and swallowing, and respiratory or cardiovascular collapse.[42]

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.[43] The toxins are potent inhibitors of liver protein phosphatase type 1 and are capable of causing massive hepatic hemorrhage and death.[43]

One of the more potent toxins in this family, microcystin-LR, has a parenteral LD_{50} of 30 to 200 µg/kg in rodents.[34] Exposure to microcystin-LR causes animals to become listless and prone in the cage; death occurs in 16 to 24 h. The toxic effects of microcystin vary depending upon the route of exposure and may include hypotension and cardiogenic shock, in addition to hepatotoxicity.[34,44]

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. Metabolites of several marine toxins, including saxitoxin, tetrodotoxin, and brevetoxins, are well-studied as part of routine regulation of food supplies.[36] Likewise, T-2 mycotoxin absorption and biodistribution has been studied, and its metabolites can be detected as late as 28 days after exposure.[27] Pathologic specimens include blood, urine, lung, liver, and stomach contents. Environmental and clinical samples can be tested using a gas liquid chromatography-mass spectrometry technique.

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/chemical stability of the LMW toxins contribute 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.[45]

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.

A BSC (Class II, Type B1 or B2) or a chemical fume hood equipped with exhaust HEPA filters and a charcoal filter are indicated for activities with a high potential for aerosol, such as powder samples, and the use of large quantities of toxin. Laboratory coat and gloves should be worn if potential skin contact exists. The use of respiratory protection should be considered if potential aerosolization of toxin exists.

For LMW toxins that are not easily decontaminated with bleach solutions, it is recommended to use pre-positioned, disposable liners for laboratory bench 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.[46]

Select Agent Some LMW toxins are a select agent 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 this agent may require CDC and/or USDA 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.

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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 (Tables 7 and 8).[1,2] A central biochemical feature of prion diseases is the conversion of normal prion protein (PrP) to an abnormal, misfolded, pathogenic isoform designated PrP^{Sc} (named for "scrapie," the prototypic prion disease). The infectious agents that transmit prion diseases are resistant to inactivation by heat and chemicals and thus require special biosafety precautions. Prion diseases are transmissible by inoculation or ingestion of infected tissues or homogenates, and infectivity is present at high levels in brain or other central nervous system tissues, and at slightly lower levels in lymphoid tissues including spleen, lymph nodes, gut, bone marrow, and blood. Although the biochemical nature of the infectious TSE agent, or prion, is not yet proven, the infectivity is strongly associated with the presence of PrP^{Sc}, suggesting that this material may be a major component of the infectious agent.

A chromosomal gene encodes PrP^{C} (the cellular isoform of PrP) and no PrP genes are found in purified preparations of prions. PrP^{Sc} is derived from PrP^{C} by a posttranslational process whereby PrP^{Sc} acquires a high *beta*-sheet content and a resistance to inactivation by normal disinfection processes. The PrPSc is less soluble in aqueous buffers and, when incubated with protease (proteinase K), the PrP^{C} is completely digested (sometimes indicated by the "sensitive" superscript, PrP^{sen}) while PrP^{Sc} is resistant to protease (PrP^{res}). Neither PrP-specific nucleic acids nor virus-like particles have been detected in purified, infectious preparations.

Occupational Infections

No occupational infections have been recorded from working with prions. No increased incidence of Creutzfeldt-Jakob disease (CJD) has been found amongst pathologists who encounter cases of the disease post-mortem.

Natural Modes of Infection

The recognized diseases caused by prions are listed under Table 7 (human diseases) and Table 8 (animal diseases). The only clear risk factor for 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 and related diseases such as feline spongiform encephalopathy. It is also possible to acquire certain diseases such as familial CJD by inheritance through the germ line.

Most TSE agents, or prions, have a preference for infection of 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; however, infectivity for the original host may also be propagated for several passages over a time-span of years. The process of cross-species adaptation can also vary among individuals in the same species and the rate of adaptation and the final species specificity is difficult to predict with accuracy. Such considerations help to form the basis for the biosafety classification of different prions.

Table 7. The Human Prion Diseases

Disease	Abbreviation	Mechanism of Pathogenesis
Kuru		Infection through ritualistic cannibalism
Creutzfeldt-Jakob disease	CJD	Unknown mechanism
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 and secondary bloodborne transmission
Familial CJD	fCJD	Germline mutations in PrP gene
Latrogenic CJD	iCJD	Infection from contaminated corneal and dural grafts, pituitary hormone, or neurosurgical equipment
Gerstmann-Sträussler-Scheinker syndrome	GSS	Germline mutations in PrP gene
Fatal familial insomnia	FFI	Germline mutations in PrP gene

Table 8. The Animal Prion Diseases

Disease	Abbreviation	Natural Host	Mechanism of Pathogenesis
Scrapie		Sheep, goats, mouflon	Infection in genetically susceptible sheep
Bovine spongiform encephalopathy	BSE	Cattle	Infection with prion-contaminated feedstuffs
Chronic wasting disease	CWD	Mule, deer, white-tailed deer, Rocky Mountain elk	Unknown mechanism; possibly from direct animal contact or indirectly from contaminated feed and water sources
Exotic ungulate encephalopathy	EUE	Nyala, greater kudu and oryx	Infection with BSE-contaminated feedstuffs
Feline spongiform encephalopathy	FSE	Domestic and wild cats in captivity	Infection with BSE-contaminated feedstuffs
Transmissible mink encephal-opathy	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 should be manipulated at BSL-2. BSE prions can likewise be manipulated at BSL-2. Due to the high probability that BSE prions have been transmitted to humans, certain circumstances may require the use of BSL-3 facilities and practices. All other animal prions are manipulated at BSL-2. However, when a prion from one species is inoculated into another the resultant infected animal should be treated according to the guidelines applying to the source of the inoculum. Contact APHIS National Center for Import and Export at (301) 734-5960 for specific guidance.

Although the exact mechanism of spread of scrapie among sheep and goats developing natural scrapie is unknown, there is considerable evidence that one of the primary sources is oral inoculation with placental membranes from infected ewes. There has been no evidence for transmission of scrapie to humans, even though the disease was recognized in sheep for over 200 years. The diseases TME, BSE, FSE, and EUE are all thought to occur after the consumption of prion-infected foods.[1,2] The exact mechanism of CWD spread among mule deer, white-tailed deer and Rocky Mountain elk is unknown. There is strong evidence that CWD is laterally transmitted and environmental contamination may play an important role in local maintenance of the disease.[2]

In the care of patients diagnosed with human prion disease, Standard Precautions are adequate. However, the human prion diseases in this setting

are not communicable or contagious.[3] There is no evidence of contact or aerosol transmission of prions from one human to another. However, they are infectious under some circumstances, such as ritualistic cannibalism in New Guinea causing kuru, the administration of prion-contaminated growth hormone causing iatrogenic CJD, and the transplantation of prion-contaminated dura mater and corneal grafts. It is highly suspected that variant CJD can also be transmitted by blood transfusion.[4] However, there is no evidence for bloodborne transmission of non-variant forms of CJD. Familial CJD, GSS, and FFI are all dominantly inherited prion diseases; many different mutations of the PrP gene have been shown to be genetically linked to the development of inherited prion disease. Prions from many cases of inherited prion disease have been transmitted to apes, monkeys, and mice, especially those carrying human PrP transgenes.

Special Issues

Inactivation of Prions Prions are characterized by resistance to conventional inactivation procedures including irradiation, boiling, dry heat, and chemicals (formalin, betapropiolactone, alcohols). While prion infectivity in purified samples is diminished by prolonged digestion with proteases, results from boiling in sodium dodecyl sulfate and urea are variable. Likewise, denaturing organic solvents such as phenol or chaotropic reagents such as guanidine isothiocyanate have also resulted in greatly reduced but not complete inactivation. The use of conventional autoclaves as the sole treatment has not resulted in complete inactivation of prions.[5] Formalin-fixed and paraffin-embedded tissues, especially of the brain, remain infectious. Some investigators recommend that formalin-fixed tissues from suspected cases of prion disease be immersed for 30 min in 96% formic acid or phenol before histopathologic processing (Table 9), but such treatment may severely distort the microscopic neuropathology.

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.[6] Current recommendations for inactivation of prions on instruments and other materials are based on the use of sodium hypochlorite, NaOH, Environ LpH and the moist heat of autoclaving with combinations of heat and chemical being most effective (Table 9).[5,6]

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.[6] Sterilization of reusable surgical instruments and decontamination of surfaces should be performed in accordance with recommendations described by the CDC (*www.cdc.gov*) and the WHO infection control guidelines.[6] Table 9 summarizes the key recommendations for decontamination of reusable instruments and surfaces. Contaminated disposable instruments or materials should be incinerated at 1000° C or greater.[7]

Autopsies Routine autopsies and the processing of small amounts of formalin-fixed tissues containing human prions can safely be done using Standard Precautions.[8] 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 spleen, thymus, lymph nodes, 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.[3] Persons handling contaminated specimens should wear cut-resistant gloves if possible. If accidental contamination of unbroken skin occurs, the area should be washed with detergent and abundant quantities of warm water (avoid scrubbing); brief exposure (1 minute to 1N NaOH or a 1:10 dilution of bleach) can be considered for maximum safety.[6] Additional guidance related to occupational injury are provided in the WHO infection control guidelines.[6] Unfixed samples of brain, spinal cord, and other tissues containing human prions should be processed with extreme care in a BSL-2 facility utilizing BSL-3 practices.

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.[9] The most prudent approach is to study BSE prions at a minimum in a BSL-2 facility utilizing 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 should wear full body coverage personal protective equipment (e.g., gloves, rear closing gown and face shield). Disposable plasticware, which can be discarded as a dry regulated medical waste, is highly recommended. Because the paraformaldehyde vaporization procedure does not diminish prion titers, BSCs must be decontaminated with 1N NaOH and rinsed with water. HEPA filters should be bagged out and incinerated. Although there is no evidence to suggest that aerosol transmission occurs in the natural disease, it is 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).[6] 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 pressurized vessel that exposes the carcass or tissues to 1 N NaOH or KOH heated to 150°C, can be used as an alternative to incineration for the disposal of carcasses and tissue.[5,10] The process has been shown to completely inactive TSEs (301v agent used) when used for the recommended period.

Table 9. Tissue Preparation for Human CJD and Related Diseases

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 is collected in a 4L waste bottle initially containing 600 ml 6N NaOH.
4.	Gloves, embedding molds, and all handling materials are disposed s regulated medical waste.
5.	Tissue cassettes are processed manually to prevent contamination of tissue processors.
6.	Tissues are embedded in a disposable embedding mold. If used, forceps are decontaminated as in Table 10.
7.	In preparing sections, gloves are worn, section waste is collected and disposed in a regulated medical waste receptacle. The knife stage is wiped with 2N NaOH, and 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; b. reagents are prepared in 100 ml disposable specimen cups; c. after placing the cover slip on, slides are decontaminated by soaking them for 1 hour in 2N NaOH; d. 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; 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 particular attention to the facilities, equipment, policies, and procedures involved.[10] The related considerations outlined in Table 9 should be incorporated into the laboratory's risk management for this work.

Table 10. Prion Inactivation Methods for Reusable Instruments and Surfaces

1.	Immerse in 1 N NaOH, heat in a gravity displacement autoclave at 121ºC for 30 minutes. Clean and sterilize by conventional means.
2.	Immerse in 1 N NaOH or sodium hypochlorite (20,000 ppm) for 1 hours. Transfer into water and autoclave (gravity displacement) at 121ºC for 1 hour. Clean and sterilize by conventional means.
3.	Immerse in 1N NaOH or sodium hypochlorite (20,000) for 1 hour. 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 2N 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.	Environ LpH (EPA Reg. No. 1043-118) 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.

(Adapted from *www.cdc.gov* [11,12])

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 fresh 1:10 dilutions (1 part 10 N NaOH plus 9 parts water) used daily.

20,000 ppm sodium hypochlorite equals a 2% solution. Most commercial household bleach contains 5.25% sodium hypochlorite, therefore, make a 1:2.5 dilution (1 part 5.25% bleach plus 1.5 parts water) to produce a 20,000 ppm solution. This ratio can also be stated as two parts 5.25% bleach to three parts water. Working solutions should be prepared daily.

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.

Precautions in 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. 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. Immersion in sodium hypochlorite bleach can cause severe damage to some instruments.

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Appendix A – Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets

Section I—Introduction

This document presents information on the design, selection, function and use of Biological Safety Cabinets (BSCs), which are the primary means of containment developed for working safely with infectious microorganisms. Brief descriptions of the facility and engineering concepts for the conduct of microbiological research are also provided. BSCs are only one part of an overall biosafety program, which requires consistent use of good microbiological practices, use of primary containment equipment and proper containment facility design. Detailed descriptions of acceptable work practices, procedures and facilities, known as Biosafety Levels 1 through 4, are presented in the CDC/NIH publication Biosafety in Microbiological and Biomedical Laboratories (BMBL).[1]

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets, designated as Class I, II and III, have been developed to meet varying research and clinical needs.

Most BSCs use high efficiency particulate air (HEPA) filters in the exhaust and supply systems. The exception is a Class I BSC, which does not have HEPA filtered supply air. These filters and their use in BSCs are briefly described in Section II. Section III presents a general description of the special features of BSCs that provide varying degrees of personnel, environmental, and product protection.

Laboratory hazards and risk assessment are discussed in Section IV. Section V presents work practices, procedures and practical tips to maximize information regarding the protection afforded by the most commonly used BSCs. Facility and engineering requirements needed for the operation of each type of BSC are presented in Section VI. Section VII reviews requirements for routine annual certification of cabinet operation and integrity.

These sections are not meant to be definitive or all encompassing. Rather, an overview is provided to clarify the expectations, functions and performance of these critical primary barriers. This document has been written for the biosafety officer, laboratorian, engineer or manager who desires a better understanding of each type of cabinet; factors considered for the selection of a BSC to meet specific operational needs; and the services required to maintain the operational integrity of the cabinet.

Proper maintenance of cabinets used for work at all biosafety levels cannot be over emphasized. Biosafety Officers (BSOs) should understand that an active cabinet is a primary containment device. A BSC must be routinely inspected and tested by training personnel, following strict protocols, to verify that it is working

properly. This process is referred to as certification of the cabinet and should be performed annually.

Section II—The High Efficiency Particulate Air (HEPA) Filter and the Development of Biological Containment Devices

From the earliest laboratory-acquired typhoid infections to the hazards posed by bioterrorism, antibiotic-resistant bacteria and rapidly mutating viruses, threats to worker safety have stimulated the development and refinement of workstations in which infectious microorganisms could be safely handled. The needs to work with tissue cultures, maintain sterility of cell lines, and minimize cross-contamination have contributed to concerns regarding product integrity.

The use of proper procedures and equipment (as described in BMBL)[1] cannot be overemphasized in providing primary personnel and environmental protection. For example, high-speed blenders designed to reduce aerosol generation, needle-locking syringes, micro burners and safety centrifuge cups or sealed rotors are among the engineered devices that protect laboratory workers from biological hazards. An important piece of safety equipment is the biological safety cabinet in which manipulations of infectious microorganisms are performed.

Background

Early prototype clean air cubicles were designed to protect the materials being manipulated from environmental or worker-generated contamination rather than to protect the worker from the risks associated with the manipulation of potentially hazardous materials. Filtered air was blown across the work surface directly at the worker. Therefore, these cubicles could not be used for handling infectious agents because the worker was in a contaminated air stream.

To protect the worker during manipulations of infectious agents, a small workstation was needed that could be installed in existing laboratories with minimum modification to the room. The earliest designs for primary containment devices were essentially non-ventilated “boxes” built of wood and later of stainless steel, within which simple operations such as weighing materials could be accomplished.[2]

Early versions of ventilated cabinets did not have adequate or controlled directional air movement. They were characterized by mass airflow into the cabinets albeit with widely varying air volumes across openings. Mass airflow into cabinet drew “contaminated” air away from the laboratory worker. This was the forerunner of the Class I BSC. However, since the air was unfiltered, the cabinet was contaminated with environmental microorganisms and other undesirable particulate matter.

Control of airborne particulate materials became possible with the development of filters, which efficiently removed microscopic contaminants from the air. The HEPA filter was developed to create dust-free work environments (e.g., “clean rooms” and “clean benches”) in the 1940s.[2]

HEPA filters remove the most penetrating particle size (MPPS) of 0.3 µm with an efficiency of at least 99.97%. Particles both larger and smaller than the MPPS are removed with greater efficiency. Bacteria, spores and viruses are removed from the air by these filters. HEPA filter efficiency and the mechanics of particle collection by these filters have been studied and well-documented [3,4] therefore only a brief description is included here.

The typical HEPA filter medium is a single sheet of borosilicate fibers treated with a wet-strength water-repellant binder. The filter medium is pleated to increase the overall surface area inside the filter frames and the pleats are often divided by corrugated aluminum separators (Figure 1). The separators prevent the pleats from collapsing in the air stream and provide a path for airflow. Alternate designs providing substitutions for the aluminum separators may also be used. The filter is glued into a wood, metal or plastic frame. Careless handling of the filter (e.g., improper storage or dropping) can damage the medium at the glue joint and cause tears or shifting of the filter resulting in leaks in the medium. This is the primary reason why filter integrity must be tested when a BSC is installed initially and each time it is moved or relocated. (See Section VII.)

Various types of containment and clean air devices incorporate the use of HEPA filters in the exhaust and/or supply air system to remove airborne particulate material. Depending on the configuration of these filters and the direction of the airflow, varying degrees of personnel, environmental and product protection can be achieved.[5] Section V describes the proper practices and procedures necessary to maximize the protection afforded by the device.

Section III—Biological Safety Cabinets

The similarities and differences in protection offered by the various classes of BSCs are reflected in Table 1. Please also refer to Table 2 and Section IV for further considerations pertinent to BSC selection and risk assessment.

The Class I BSC

The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in terms of air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment (Figure 2). In the Class I BSC, unfiltered room air is drawn in through the work opening and across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfm) is maintained[6] through the front opening. Because product protection is provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases, Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures with potential to generate aerosols (e.g., cage dumping, culture aeration or tissue homogenation).

The classical Class I BSC is hard-ducted (i.e., direct connection) to the building exhaust system and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the cabinet exhaust plenum. A second HEPA filter may be installed at the terminal end of the building exhaust system prior to the exhaust fan.

Some Class I BSCs are equipped with an integral exhaust fan. The cabinet exhaust fan must be interlocked with the building exhaust fan. In the event that the building exhaust fan fails, the cabinet exhaust fan must turn off so that the building exhaust ducts are not pressurized. If the ducts are pressurized and the HEPA filter has developed a leak, contaminated air could be discharged into other parts of the building or the environment. The use of two filters in the cabinet increases the static pressure on the fan.

A panel with openings to allow access for the hands and arms to the work surface can be added to the Class I cabinet. The restricted opening results in increased inward air velocity, increasing worker protection. For added safety, arm-length gloves can be attached to the panel. Makeup air is then drawn through an auxiliary air supply opening (which may contain a filter) and/or around a loose-fitting front panel.

Some Class I models used for animal cage changing are designed to allow recirculation of air into the room after HEPA filtration and may require more frequent filter replacement due to filter loading and odor from organic materials captured on the filter. This type of Class I BSC should be certified annually for sufficient airflow and filter integrity.

The Class II BSC

As biomedical researchers began to use sterile animal tissue and cell culture systems, particularly for the propagation of viruses, cabinets were needed that also provided product protection. In the early 1960s, the “laminar flow” principle evolved. Unidirectional air moving at a fixed velocity along parallel lines was demonstrated to reduce turbulence resulting in predictable particle behavior. Biocontainment technology also incorporated this laminar flow principle with the use of the HEPA filter to aid in the capture and removal of airborne contaminants from the air stream.[7] This combination of technologies serves to help protect the laboratory worker from potentially infectious aerosols[4] generated within the cabinet and provides necessary product protection, as well. Class II BSCs are partial barrier systems that rely on the directional movement of air to provide containment. As the air curtain is disrupted (e.g., movement of materials in and out of a cabinet, rapid or sweeping movement of the arms) the potential for contaminant release into the laboratory work environment is increased, as is the risk of product contamination.

The Class II (Types A1, A2, B1 and B2)[8] BSCs provide personnel, environmental and product protection. Airflow is drawn into the front grille of

the cabinet, providing personnel protection. In addition, the downward flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Because cabinet exhaust air is passed through a certified HEPA filter, it is particulate-free (environmental protection), and may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy or "thimble" connected to the building exhaust. Exhaust air from Types B1 and B2 BSCs must be discharged directly to the outdoors via a hard connection.

HEPA filters are effective at trapping particulates and thus infectious agents but do not capture volatile chemicals or gases. Only Type A2-exhausted or Types B1and B2 BSCs exhausting to the outside should be used when working with volatile, toxic chemicals, but amounts must be limited (Table 2).

All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2, 3 and 4.[1] Class II BSCs provide the microbe-free work environment necessary for cell culture propagation and also may be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs.[9] Class II BSCs may be used with organisms requiring BSL-4 containment in a BSL-4 suit laboratory by a worker wearing a positive pressure protective suit.

1. *The Class II, Type A1 BSC:* An internal fan (Figure 3) draws sufficient room air through the front grille to maintain a minimum calculated or measured average inflow velocity of at least 75 lfm at the face opening of the cabinet. The supply air flows through a HEPA filter and provides particulate-free air to the work surface. Airflow provided in this manner reduces turbulence in the work zone and minimizes the potential for cross-contamination.

   The downward moving air "splits" as it approaches the work surface; the fan[6] draws part of the air to the front grille and the remainder to the rear grille. Although there are variations among different cabinets, this split generally occurs about halfway between the front and rear grilles and two to six inches above the work surface.

   The air is drawn through the front and rear grilles by a fan pushed into the space between the supply and exhaust filters. Due to the relative size of these two filters, approximately 30% of the air passes through the exhaust HEPA filter and 70% recirculates through the supply HEPA filter back into the work zone of the cabinet. Most Class II, Type A1 and A2 cabinets have dampers to modulate this division of airflow.

   A Class II Type A1 BSC is not to be used for work involving volatile toxic chemicals. The buildup of chemical vapors in the cabinet (by recirculated air) and in the laboratory (from exhaust air) could create health and safety hazards (See Section IV).

It is possible to exhaust the air from a Type A1 or A2 cabinet outside of the building. However, it must be done in a manner that does not alter the balance of the cabinet exhaust system, thereby disturbing the internal cabinet airflow. The proper method of connecting a Type A1 or A2 cabinet to the building exhaust system is through use of a canopy hood,[8,10] which provides a small opening or air gap (usually 1 inch) around the cabinet exhaust filter housing (Figure 4). The airflow of the building exhaust must be sufficient to maintain the flow of room air into the gap between the canopy unit and the filter housing. The canopy must be removable or be designed to allow for operational testing of the cabinet. (See Section VI.) Class II Type A1 or A2 cabinets should never be hard-ducted to the building exhaust system.[8] Fluctuations in air volume and pressure that are common to all building exhaust systems sometimes make it difficult to match the airflow requirements of the cabinet.

2. *The Class II, Type B1 BSC:* Some biomedical research requires the use of small quantities of hazardous chemicals, such as organic solvents or carcinogens. Carcinogens used in cell culture or microbial systems require both biological and chemical containment.[11]

   The Class II, Type B cabinet originated with the National Cancer Institute (NCI)-designed Type 212 (later called Type B) BSC (Figure 5A), and was designed for manipulations of minute quantities of hazardous chemicals with *in vitro* biological systems. The NSF International NSF/ANSI Standard 49—2007 definition of Type B1 cabinets[8] includes this classic NCI design Type B, and cabinets without supply HEPA filters located immediately below the work surface (Figure 5B), and/or those with exhaust/recirculation down flow splits other than exactly 70/30%.

   The cabinet supply blowers draw room air (plus a portion of the cabinet's recirculated air) through the front grille and through the supply HEPA filters located immediately below the work surface. This particulate-free air flows upward through a plenum at each side of the cabinet and then downward to the work area through a backpressure plate. In some cabinets, there is an additional supply HEPA filter to remove particulates that may be generated by the blower-motor system.

   Room air is drawn through the face opening of the cabinet at a minimum measured inflow velocity of 100 lfm. As with the Type A1 and A2 cabinets, there is a split in the down-flowing air stream just above the work surface. In the Type B1 cabinet, approximately 70 percent of the down flow air exits through the rear grille, passes through the exhaust HEPA filter, and is discharged from the building. The remaining 30 percent of the down flow air is drawn through the front grille. Since the air that flows to the rear grille is discharged into the exhaust system, activities that may

generate hazardous chemical vapors or particulates should be conducted toward the rear of the cabinetwork area.[13]

Type B1 cabinets must be hard-ducted, preferably to a dedicated, independent exhaust system. As indicated earlier, fans for laboratory exhaust systems should be located at the terminal end of the ductwork to avoid pressuring the exhaust ducts. A failure in the building exhaust system may not be apparent to the user, as the supply blowers in the cabinet will continue to operate. A pressure-independent monitor and alarm should be installed to provide warning and shut off the BSC supply fan, should failure in exhaust airflow occur. Since this feature is not supplied by all cabinet manufacturers, it is prudent to install a sensor such as a flow monitor and alarm in the exhaust system as necessary. To maintain critical operations, laboratories using Type B1 BSCs should connect the exhaust blower to the emergency power supply.

3. *The Class II, Type B2 BSC:* This BSC is a total-exhaust cabinet; no air is recirculated within it (Figure 6). This cabinet provides simultaneous primary biological and chemical (small quantity) containment. Consideration must be given to the chemicals used in BSCs as some chemicals can destroy the filter medium, housings and/or gaskets causing loss of containment. The supply blower draws either room or outside air in at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet. The building exhaust system draws air through both the rear and front grills, capturing the supply air plus the additional amount of room air needed to produce a minimum calculated or measured inflow face velocity of 100 lfm. All air entering this cabinet is exhausted, and passes through a HEPA filter (and perhaps some other air-cleaning device such as a carbon filter if required for the work being performed) prior to discharge to the outside. This cabinet exhausts as much as 1200 cubic feet per minute of conditioned room air making this cabinet expensive to operate. The higher static air pressure required to operate this cabinet also results in additional costs associated with heavier gauge ductwork and higher capacity exhaust fan. Therefore, the need for the Class II, Type B2 should be justified by the research to be conducted.

   Should the building exhaust system fail, the cabinet will be pressurized, resulting in a flow of air from the work area back into the laboratory. Cabinets built since the early 1980's usually have an interlock system, installed by the manufacturer, to prevent the supply blower from operating whenever the exhaust flow is insufficient; systems can be retrofitted if necessary. Exhaust air movement should be monitored by a pressure-independent device, such as a flow monitor.

4. *The Class II, Type A2 BSC (Formerly called A/B3):* Only when this BSC (Figure 7) is ducted to the outdoors does it meet the requirements of the

former Class II Type B3.[8] The Type A2 cabinet has a minimum calculated or measured inflow velocity of 100 lfm. All positive pressure contaminated plenums within the cabinet are surrounded by a negative air pressure plenum thus ensuring that any leakage from a contaminated plenum will be drawn into the cabinet and not released to the environment. Minute quantities of volatile toxic chemicals or radionuclides can be used in a Type A2 cabinet only if it exhausts to the outside via a properly functioning canopy connection.[8]

5. *Special Applications:* Class II BSCs can be modified to accommodate special tasks. For example, the front sash can be modified by the manufacturer to accommodate the eyepieces of a microscope. The work surface can be designed to accept a carboy, a centrifuge or other equipment that may require containment. A rigid plate with openings for the arms can be added if needed. Good cabinet design, microbiological aerosol tracer testing of the modification and appropriate certification (see Section VII) are required to ensure that the basic systems operate properly after modification. Maximum containment potential is achieved only through strict adherence to proper practices and procedures (see Section V).

The Class III BSC

The Class III BSC (Figure 8) was designed for work with highly infectious microbiological agents and for the conduct of hazardous operations and provides maximum protection for the environment and the worker. It is a gas-tight (no leak greater than 1x10-7 cc/sec with 1% test gas at 3 inches pressure Water Gauge[14]) enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank, that is accessible through the cabinet floor, or double-door pass-through box (e.g., an autoclave) that can be decontaminated between uses. Reversing that process allows materials to be removed from the Class III BSC safely. Both supply and exhaust air are HEPA filtered on a Class III cabinet. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge directly to the outdoors. Class III cabinets are not exhausted through the general laboratory exhaust system. Airflow is maintained by an exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (minimum of 0.5 inches of water gauge.)

Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet to allow direct manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials. The trade-off is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment. Laminar airflow is not a characteristic of a Class III cabinet.

Several Class III BSCs can be joined together in a "line" to provide a larger work area. Such cabinet lines are custom-built; the equipment installed in the cabinet line (e.g., refrigerators, small elevators, shelves to hold small animal cage racks, microscopes, centrifuges, incubators) is generally custom-built as well.

Horizontal Laminar Flow "Clean Bench"

Horizontal laminar flow "clean benches" (Figure 9A) are not BSCs. These pieces of equipment discharge HEPA-filtered air from the back of the cabinet across the work surface and toward the user. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. Clean benches should never be used when handling cell culture materials, drug formulations, potentially infectious materials, or any other potentially hazardous materials. The worker will be exposed to the materials being manipulated on the clean bench potentially resulting in hypersensitivity, toxicity or infection depending on the materials being handled. Horizontal airflow "clean benches" must never be used as a substitute for a biological safety cabinet. Users must be aware of the differences between these two devices.

Vertical Flow "Clean Bench"

Vertical flow clean benches (Figure 9B) also are not BSCs. They may be useful, for example, in hospital pharmacies when a clean area is needed for preparation of intravenous solutions. While these units generally have a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems presented by the horizontal laminar flow clean benches. These benches should never be used for the manipulation of potentially infectious or toxic materials or for preparation of antineoplastic agents.

Section IV—Other Laboratory Hazards and Risk Assessment

Primary containment is an important strategy in minimizing exposure to the many chemical, radiological and biological hazards encountered in the laboratory. An overview is provided, in Table 2, of the various classes of BSCs, the level of containment afforded by each and the appropriate risk assessment considerations. Microbiological risk assessment is addressed in depth in BMBL.[1]

Working with Chemicals in BSCs

Work with infectious microorganisms often requires the use of various chemical agents, and many commonly used chemicals vaporize easily. Therefore, evaluation of the inherent hazards of the chemicals must be part of the risk assessment when selecting a BSC. Flammable chemicals should not be used in Class II, Type A1 or A2 cabinets since vapor buildup inside the cabinet presents a fire hazard. In order to determine the greatest chemical concentration, which might be entrained in the air stream following an accident or spill, it is necessary

to evaluate the quantities to be used. Mathematical models are available to assist in these determinations.[13] For more information regarding the risks associated with exposure to chemicals, the reader should consult the Threshold Limit Values (TLVs) for various chemical substances established by the American Conference of Governmental Industrial Hygienists.[15]

The electrical systems of Class II BSCs are not spark-proof. Therefore, a chemical concentration approaching the lower explosive limits of the compound must be prohibited. Furthermore, since non-exhausted Class II, Type A1 and A2 cabinets return chemical vapors to the cabinetwork space and the room, they may expose the operator and other room occupants to toxic chemical vapors.

A chemical fume hood should be used for procedures using volatile chemicals instead of a BSC. Chemical fume hoods are connected to an independent exhaust system and operate with single-pass air discharged, directly or through a manifold, outside the building. They may also be used when manipulating chemical carcinogens.[11] When manipulating small quantities of volatile toxic chemicals required for use in microbiological studies, Class I and Class II (Type B2) BSCs, exhausted to the outdoors, can be used. The Class II, Type B1 and A2 canopy-exhausted cabinets may be used with minute or tracer quantities of nonvolatile toxic chemicals.[8]

Many liquid chemicals, including nonvolatile antineoplastic agents, chemotherapeutic drugs and low-level radionuclides, can be safely handled inside Class II, Type A cabinets.[9] Class II BSCs should not be used for labeling of biohazardous materials with radioactive iodine. Hard-ducted, ventilated containment devices incorporating both HEPA and charcoal filters in the exhaust systems are necessary for the conduct of this type of work (Figure 10).

Many virology and cell culture laboratories use diluted preparations of chemical carcinogens[11,16] and other toxic substances. Prior to maintenance, careful evaluation must be made of potential problems associated with decontaminating the cabinet and the exhaust system. Air treatment systems, such as a charcoal filter in a bag-in/bag-out housing,[17] (Figure 13) may be required so that discharged air meets applicable emission regulations.

National Sanitation Foundation (NSF)/ANSI Standard 49—2007[8] requires biologically-contaminated ducts and plenums of Class II, Type A2 and B cabinets be maintained under negative air pressure, or surrounded by negative pressure ducts and plenums.

Radiological Hazards in the BSC

As indicated above, volatile radionuclides such as I^{125} should not be used within Class II BSCs. When using nonvolatile radionuclides inside a BSC, the same hazards exist as if working with radioactive materials on the bench top. Work that has the potential for splatter or creation of aerosols can be done within the BSC.

Radiologic monitoring must be performed. A straight, vertical (not sloping) beta shield may be used inside the BSC to provide worker protection. A sloping shield can disrupt the air curtain and increase the possibility of contaminated air being released from the cabinet. A radiation safety professional should be contacted for specific guidance.

Risk Assessment

The potential for adverse events must be evaluated to eliminate or reduce to the greatest extent possible worker exposure to infectious organisms and to prevent release to the environment. Agent summary statements detailed in BMBL[1] provide data for microorganisms known to have caused laboratory-associated infections that may be used in protocol-driven risk assessment. Through the process of risk assessment, the laboratory environment and the work to be conducted are evaluated to identify hazards and develop interventions to ameliorate risks.

A properly certified and operational BSC is an effective engineering control (see Section VI) that must be used in concert with the appropriate practices, procedures and other administrative controls to further reduce the risk of exposure to potentially infectious microorganisms. Suggested work practices and procedures for minimizing risks when working in a BSC are detailed in the next section.

Section V — BSC Use by the Investigator: Work Practices and Procedures

Preparing for Work Within a Class II BSC

Preparing a written checklist of materials necessary for a particular activity and placing necessary materials in the BSC before beginning work serves to minimize the number and extent of air curtain disruptions compromising the fragile air barrier of the cabinet. The rapid movement of a worker's arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and compromise the partial containment barrier provided by the BSC. Moving arms in and out slowly, perpendicular to the face opening of the cabinet will reduce this risk. Other personnel activities in the room (e.g., rapid movements near the face of the cabinet, walking traffic, room fans, open/closing room doors) may also disrupt the cabinet air barrier.[6]

Laboratory coats should be worn buttoned over street clothing; latex, vinyl, nitrile or other suitable gloves are worn to provide hand protection. Increasing levels of PPE may be warranted as determined by an individual risk assessment. For example, a solid front, back-closing laboratory gown provides better protection of personal clothing than a traditional laboratory coat and is a recommended practice at BSL-3.

Before beginning work, the investigator should adjust the stool height so that his/her face is above the front opening. Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the

cabinet. This allows the cabinet to stabilize, to “air sweep” the hands and arms, and to allow time for turbulence reduction. When the user’s arms rest flatly across the front grille, occluding the grille opening, room air laden with particles may flow directly into the work area, rather than being drawn down through the front grille. Raising the arms slightly will alleviate this problem. The front grille must not be blocked with toweling, research notes, discarded plastic wrappers, pipetting devices, etc. All operations should be performed on the work surface at least four inches in from the front grille. If there is a drain valve under the work surface, it should be closed prior to beginning work in the BSC.

Materials or equipment placed inside the cabinet may cause disruption of the airflow, resulting in turbulence, possible cross-contamination and/or breach of containment. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet. Only the materials and equipment required for the immediate work should be placed in the BSC.

BSCs are designed for 24-hour per day operation and some investigators find that continuous operation helps to control the laboratory’s level of dust and other airborne particulates. Although energy conservation may suggest BSC operation only when needed, especially if the cabinet is not used routinely, room air balance is an overriding consideration. Air discharged through ducted BSCs must be considered in the overall air balance of the laboratory.

If the cabinet has been shut down, the blowers should be operated at least four minutes before beginning work to allow the cabinet to “purge.” This purge will remove any suspended particulates in the cabinet. The work surface, the interior walls (except the supply filter diffuser), and the interior surface of the window should be wiped with 70% ethanol (EtOH), a 1:100 dilution of household bleach (i.e., 0.05% sodium hypochlorite), or other disinfectant as determined by the investigator to meet the requirements of the particular activity. When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces. Wiping with non-sterile water may recontaminate cabinet surfaces, a critical issue when sterility is essential (e.g., maintenance of cell cultures).

Similarly, the surfaces of all materials and containers placed into the cabinet should be wiped with 70% EtOH to reduce the introduction of contaminants to the cabinet environment. This simple step will reduce introduction of mold spores and thereby minimize contamination of cultures. Further reduction of microbial load on materials to be placed or used in BSCs may be achieved by periodic decontamination of incubators and refrigerators.

Material Placement Inside the BSC

Plastic-backed absorbent toweling can be placed on the work surface but not on the front or rear grille openings. The use of toweling facilitates routine cleanup

and reduces splatter and aerosol generation[19] during an overt spill. It can be folded and placed in a biohazard bag or other appropriate receptacle when work is completed.

All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet (Figure 11). Similarly, aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet to take advantage of the air split described in Section III. Bulky items such as biohazard bags, discard pipette trays and vacuum collection flasks should be placed to one side of the interior of the cabinet. If placing those items in the cabinet requires opening the sash, make sure that the sash is returned to its original position before work is initiated. The correct sash position (usually 8″ or 10″ above the base of the opening) should be indicated on the front of the cabinet. On most BSCs, an audible alarm will sound if the sash is in the wrong position while the fan is operating.

Certain common practices interfere with the operation of the BSC. The biohazard collection bag should not be taped to the outside of the cabinet. Upright pipette collection containers should not be used in BSCs nor placed on the floor outside the cabinet. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. Only horizontal pipette discard trays containing an appropriate chemical disinfectant should be used within the cabinet. Furthermore, potentially contaminated materials should not be brought out of the cabinet until they have been surface decontaminated. Alternatively, contaminated materials can be placed into a closable container for transfer to an incubator, autoclave or another part of the laboratory.

Operations Within a Class II BSC

Laboratory Hazards

Many procedures conducted in BSCs may create splatter or aerosols. Good microbiological techniques should always be used when working in a BSC. For example, techniques used to reduce splatter and aerosol generation will also minimize the potential for personnel exposure to infectious materials manipulated within the cabinet. Class II cabinets are designed so that horizontally nebulized spores introduced into the cabinet will be captured by the downward flowing cabinet air within fourteen inches[8] of travel. Therefore, as a general rule of thumb, keeping clean materials at least one foot away from aerosol-generating activities will minimize the potential for cross-contamination.

The workflow should be from “clean to dirty” (Figure 11). Materials and supplies should be placed in the cabinet in such a way as to limit the movement of “dirty” items over “clean” ones.

Several measures can be taken to reduce the chance for cross-contamination of materials when working in a BSC. Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impaction of downward air. Bottle or tube caps should not be placed on the toweling. Items should be recapped or covered as soon as possible.

Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current that prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence that disrupts the pattern of HEPA-filtered air being supplied to the work surface. When deemed absolutely necessary, touch-plate micro burners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric “furnaces” are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable or recyclable sterile loops should be used whenever possible.

Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter (Figure 12). This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to inactivate the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste.

Investigators must determine the appropriate method of decontaminating materials that will be removed from the BSC at the conclusion of the work. When chemical means are appropriate, suitable liquid disinfectant should be placed into the discard pan before work begins. Items should be introduced into the pan with minimum splatter and allowed appropriate contact time as per manufacturer’s instructions. Alternatively, liquids can be autoclaved prior to disposal. Contaminated items should be placed into a biohazard bag, discard tray, or other suitable container prior to removal from the BSC.

When a steam autoclave is used, contaminated materials should be placed into a biohazard bag or discard pan containing enough water to ensure steam generation during the autoclave cycle. The bag should be taped shut or the discard pan should be covered in the BSC prior to transfer to the autoclave. The bag should be transported and autoclaved in a leak proof tray or pan. It is a prudent practice to decontaminate the exterior surface of bags and pans just prior to removal from the cabinet.