Section VIII-A: Bacterial Agents

Bacillus anthracis

Bacillus anthracis, a Gram-positive, non-hemolytic, and non-motile bacillus, is the etiologic agent of anthrax, an acute bacterial disease among wild and domestic mammals, including humans. Like all members of the genus *Bacillus*, under adverse conditions, *B. anthracis* has the ability to produce spores that allow the organism to persist for long periods (i.e., years), withstanding heat and drying, until the return of more favorable conditions for vegetative growth.[1] It is because of this ability to produce spores coupled with significant pathogenic potential in humans that this organism is considered one of the most serious and threatening biowarfare or bioterrorism agents.[2] Most mammals are susceptible to anthrax; it mostly affects herbivores that ingest spores from contaminated soil and, to a lesser extent, carnivores that scavenge on the carcasses of diseased animals. In the United States, it occurs sporadically in animals in parts of the West, Midwest, and Southwest. Human case rates for anthrax are highest in Africa and central and southern Asia.[3] The infectious dose varies greatly from species to species and is route-dependent. The inhalation anthrax infectious dose (ID) for humans has been primarily extrapolated from inhalation challenges of non-human primates (NHPs) or studies done in contaminated wool mills. Estimates vary greatly but the median lethal dose (LD50) is likely within the range of 2,500–55,000 spores.[4] It is believed that very few spores (ten or fewer) are required for cutaneous anthrax infection.[5] Anthrax cases have been rare in the United States since the first half of the 20th century. The mortality rates have been reported to be approximately 20% for cutaneous anthrax without antibiotics, 25–75% for gastrointestinal anthrax, and 80% or more for inhalation anthrax. With treatment, <1% of cutaneous anthrax cases are fatal. The fatality rate of a series of inhalation anthrax cases in 2001 was 36% with antibiotics.[6,7] *Bacillus cereus* biovar *anthracis*, if inhaled, can produce symptoms similar to inhalation anthrax. Rapid rule-out tests to differentiate *B. cereus* biovar *anthracis* from other *Bacillus* spp. are currently not available.[6]

Occupational Infections

Occupational infections are possible when in contact with contaminated animals, animal products, or pure cultures of *B. anthracis*, and may include ranchers, veterinarians, and laboratory workers. Although numerous cases of laboratory-associated anthrax (primarily cutaneous) were reported in earlier literature, in recent years, cases of anthrax due to laboratory accidents have been rare in the United States.[8,9]

Natural Modes of Infection

The clinical forms of anthrax in humans that result from different routes of infection include:

1. Cutaneous (via broken skin);
2. Gastrointestinal (via ingestion);
3. Inhalation anthrax;[10] and
4. Injection (to date, identified in heroin-injecting drug users in northern Europe).[11,12]

Cutaneous anthrax is the most common (> 95% of human cases worldwide) and is a readily treatable form of the disease. While naturally occurring disease is no longer a significant public health problem in the United States, *B. anthracis* has become a bioterrorism concern. In 2001, 22 people were diagnosed with anthrax acquired from spores sent through the mail, including 11 cases of inhalation anthrax with five deaths and 11 cutaneous cases.[13] A report of accidental shipment of live organisms highlights the importance of adherence to handling guidelines.[14] The approach to prevention and treatment of anthrax differs from that for other bacterial infections. When selecting post-exposure prophylaxis or a combination of antimicrobial drugs for treatment of anthrax, it is recommended to consider the production of toxin, the potential for antimicrobial drug resistance, the frequent occurrence of meningitis, and the presence of latent spores.[15]

Laboratory Safety and Containment Recommendations

B. anthracis may be present in blood, skin lesion exudates, cerebrospinal fluid (CSF), pleural fluid, sputum, and rarely, in urine and feces.[12] Primary hazards to laboratory personnel are: direct and indirect contact of broken skin with cultures and contaminated laboratory surfaces, accidental parenteral inoculation and, rarely, exposure to infectious aerosols. Spores are resistant to many disinfectants and may remain viable on some surfaces for years.

BSL-3 practices, containment equipment, and facilities are recommended for work involving production quantities or high concentrations of cultures, screening environmental or unknown samples (especially powders) from anthrax-contaminated locations, diagnostics or suspected anthrax samples, and for activities with a high potential for aerosol production. As soon as *B. anthracis* is suspected in the sample, BSL-3 practices are recommended for further culture and analysis. BSL-2 practices, containment equipment, and facilities are recommended for primary inoculation of cultures from potentially infectious clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for studies utilizing experimentally infected laboratory rodents. It is recommended that all centrifugation be performed using autoclavable, aerosol-tight rotors or safety cups that are opened within the BSC after each run. In addition, it is recommended to collect routine surveillance swabs for culture inside the rotor and rotor lid and, if contaminated, it is recommended to autoclave rotors before re-use.

Special Issues

Be advised of possible misidentification using automated systems. For identification using MALDI-TOF MS, it is recommended to use alternative tube extraction that kills viable organisms in the BSC, followed by filtration through a 0.1–0.2 um filter to remove any remaining viable cells or spores, and not direct spotting of plates in the open laboratory.[15,16]

Vaccines Control of anthrax begins with control of the disease in livestock, and vaccination of livestock has long been central to control programs. Human anthrax is best controlled through prevention, including (a) pre-exposure vaccination for persons at high-risk for encountering aerosolized *B. anthracis* spores, (b) reduction of animal illness by vaccination of livestock at risk for anthrax, and (c) environmental controls to decrease exposure to contaminated animal products, such as imported hair and skins. After a person is exposed to aerosolized *B. anthracis* spores, a combination of antimicrobials and vaccine provides the best available protection.[17] A licensed vaccine for anthrax in humans is available, the anthrax vaccine adsorbed (AVA). AVA is produced from the protective antigen of an attenuated non-encapsulated strain of *B. anthracis*. The vaccine is approved by the Food and Drug Administration (FDA) for at-risk adults before exposure to anthrax. Guidelines for its use in occupational settings are available from the ACIP.[18] CDC has reviewed and updated guidelines for anthrax post-exposure prophylaxis and treatment.[17] Vaccination is not recommended for workers involved in routine processing of clinical specimens or environmental swabs in general clinical diagnostic laboratories. Of interest, Obiltoxaximab, a novel monoclonal antibody directed against the protective antigen of *B. anthracis*, which plays a key role in the pathogenesis of anthrax, has received approval for treatment and prevention of inhalational anthrax.[19] Because of the limited potential of antibiotic treatment once toxemia has already set in, numerous strategies are being explored for therapy directed against the action of anthrax toxins.[20]

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

Bordetella pertussis

Bordetella pertussis, an exclusively human respiratory pathogen of worldwide distribution, is the etiologic agent of whooping cough or pertussis. The organism

is a fastidious, small, Gram-negative coccobacillus that requires specialized culture and transport media for cultivation in the laboratory.[21] Alternatively, infection may be diagnosed by molecular methodologies on a direct specimen. Its natural habitat is the human respiratory tract.

Occupational Infections

Occupational transmission of pertussis has been reported, primarily among healthcare workers.[22] Outbreaks, including secondary transmission, among workers have been documented in hospitals, long-term care institutions, and laboratories. Nosocomial transmission has been reported in healthcare settings and laboratory-associated pertussis has also been documented.[23,24]

Natural Modes of Infection

Pertussis is highly communicable, with person-to-person transmission occurring via aerosolized respiratory secretions (droplets) containing the organism. The attack rate among susceptible hosts is affected by the frequency, proximity, and time of exposure to infected individuals; however, transmission rates to susceptible contacts may be close to 90% with the infectious dose only around 100 CFU.[21] Although the number of reported pertussis cases declined by over 99% following the introduction of vaccination programs in the 1940s, the incidence of pertussis remains cyclical, with epidemic peaks occurring every three to five years within a given region.[25] In 2015, the World Health Organization reported 142,512 pertussis cases globally and estimated that there were 89,000 deaths attributed to pertussis.[26] However, a recent publication modeling pertussis case and death estimates proposed that there were 24.1 million pertussis cases and 160,700 deaths in children younger than five years in 2014 worldwide.[27] Of significance, *B. pertussis* continues to circulate in populations despite high vaccination of infants and children because protection wanes after several years.[28]

Nevertheless, in vaccinating countries, although pertussis is primarily observed in neonates, infections are found in under-vaccinated or unvaccinated individuals of all ages, including young infants, older school children, adolescents, and adults.[27–29] Adults and adolescents with atypical or undiagnosed *B. pertussis* infections are a primary reservoir. Pertactin is an outer membrane protein and virulence factor for *B. pertussis*, and it should be noted that pertactin-negative strains may evade vaccine-mediated immunity.[30]

Laboratory Safety and Containment Recommendations

The agent may be present in high levels in respiratory secretions and may be found in other clinical material, such as blood and lung tissue.[31,32] Aerosol generation during the manipulation of cultures and contaminated clinical specimens generate the greatest potential hazard. Direct contact is also a hazard with the agent being able to survive a number of days on surfaces such as clothing.

BSL-3 practices, containment equipment, and facilities are appropriate for production operations. BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious clinical material and cultures. ABSL-2 practices and containment equipment are recommended for housing experimentally infected animals. Primary containment devices and equipment, including biological safety cabinets, safety centrifuge cups, or sealed rotors are recommended for activities likely to generate potentially infectious aerosols.

Special Issues

Vaccines A number of pertussis vaccines are available for infants, children, preteens, teens, and adults. DTaP (Diphtheria/Tetanus/Pertussis) is the childhood vaccine, and Tdap (Tetanus/Diphtheria/Pertussis) is the pertussis booster vaccine for preteens, teens, and adults.[33]

Transfer of Agent Importation of this agent requires 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.

Brucella species

The genus *Brucella* consists of slow-growing, very small, Gram-negative coccobacilli whose natural hosts are mammals. The taxonomy of *Brucella* species remains in flux; however, this genus currently includes 10 recognized species:

- Six terrestrial
  - *B. melitensis* (preferred hosts: sheep, goats, and camels)
  - *B. suis* (preferred hosts: swine and other wild animals)
  - *B. abortus* (natural hosts: cattle and buffalo)
  - *B. canis* (natural host: dogs)
  - *B. ovis* (natural host: rams)
  - *B. neotomae* (natural host: desert and wood rats)
- Three marine
  - *B. delphini*
  - *B. pinnipedialis*
  - *B. ceti*
- One proposed species of unknown origin.[34]

High-risk species for human infections include *Brucella abortus*, *B. melitensis*, and *B. suis*. There is a wide spectrum of clinical manifestations, and patients may have an extended recovery period. Mortality is estimated to be less than 1%.[34,35]

Occupational Infections

Brucellosis is a frequently reported Laboratory-associated infection.[34–38] Airborne and mucocutaneous exposures can produce Laboratory-associated infections. Many cases of laboratory-associated disease appear to be due to mishandling and misidentification of the organism.[39] The need to improve compliance with recommended guidelines was highlighted when 916 laboratory workers were exposed to the RB51 vaccine strain, which is known to cause human illness, due to mishandling of a proficiency test sample.[41] Brucellosis is an occupational disease for workers who handle infected animals or their tissues. Accidental self-inoculation with vaccine strains is an occupational hazard for veterinarians and other animal handlers.

Natural Modes of Infection

Brucellosis (Undulant fever, Malta fever, Mediterranean fever) is a zoonotic disease of worldwide occurrence. Mammals, particularly cattle, goats, swine, and sheep, act as reservoirs for *Brucella* spp. as animals are generally asymptomatic. Multiple routes of transmission have been identified, including direct contact with infected animal tissues or products, ingestion of contaminated milk, and airborne exposure in animal pens and stables.

Laboratory Safety and Containment Recommendations

Brucella may be found in a wide variety of body tissues, including blood, CSF, semen, pulmonary excretions, placenta, and occasionally urine. Most laboratory-associated cases occur in research facilities and involve exposures to zoonotic *Brucella* organisms grown in large quantities or exposure to placental tissues containing zoonotic *Brucella* spp. Cases have also occurred in clinical laboratory settings from sniffing bacteriological cultures or working on open benchtops.[42,43] Human infections are commonly attributed to exposure to aerosols or direct skin contact with cultures or infectious animal specimens.[43,44] The infectious dose of *Brucella* is 10–100 organisms by aerosol or subcutaneous routes in laboratory animals.[45,46] *Brucella* spp. are environmentally stable, surviving days to months in carcasses and organs, in soil and on surfaces.[45,46]

BSL-3 practices, containment equipment, and facilities are recommended for all manipulations of cultures of pathogenic *Brucella* spp. BSL-3 practices are recommended when handling products of conception or clinical specimens suspected to contain *Brucella*.[12] ABSL-3 practices are recommended for experimental animal studies. BSL-2 practices, containment equipment, and facilities are recommended for routine handling of clinical specimens of human or animal origin.

Special Issues

Be advised of possible misidentification using automated systems. For identification using MALDI-TOF MS, it is recommended to use alternative tube extraction that kills viable organisms and not direct spotting of plates in the open laboratory.

Vaccines Human *Brucella* vaccines have been developed and tested in other countries with limited success.[49] Although a number of successful vaccines are available for immunization of animals, no licensed human vaccines are currently available. Some recently described ribosomal proteins and fusion proteins demonstrate a protective effect against *Brucella* based on antibody and cell-mediated responses, which may prove useful in potential vaccines.[34]

Select Agent *Brucella abortus*, *B. melitensis*, and *B. suis* 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 requires 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.

Burkholderia mallei

Burkholderia mallei is a non-motile, Gram-negative rod associated with glanders, a disease primarily of equine species, but which can be seen in humans. While endemic foci of infection exist in some areas of the world, glanders due to natural infection is extremely rare in the United States with the last naturally occurring case reported in 1934.[50] Reported mortality rates are over 90% if left untreated, and up to 50% with treatment.[50]

Occupational Infections

Glanders occurs almost exclusively among individuals who work with equine species and/or handle *B. mallei* cultures in the laboratory. *B. mallei* can be very infectious in the laboratory setting. The only reported case of human glanders in the United States over the past 50 years resulted from a laboratory exposure.[51] Modes of transmission may include inhalation and/or mucocutaneous exposure.

Natural Modes of Infection

Glanders is a highly communicable disease of solipeds (such as horses, goats, and donkeys). Zoonotic transmission occurs to humans, but person-to-person transmission is rare. Glanders in solipeds and humans has been eradicated from North America and Western Europe. However, sporadic infections of animals are still reported in Far East Asia, South America, Eastern Europe, North Africa, and the Middle East.[50] Clinical manifestations in humans include localized

infection, pulmonary infection, bacteremia, or chronic infection, characterized by suppurative tissue abscesses. The organism is transmitted by direct invasion of abraded or lacerated skin; inhalation with deep lung deposition; and by bacterial invasion of the nasal, oral, and conjunctival mucous membranes. Occupational exposures most often occur through exposed skin.[50]

Laboratory Safety and Containment Recommendations

B. mallei can be hazardous in a laboratory setting. Laboratory-associated infections have resulted from aerosol and cutaneous exposure. A laboratory-associated infection in 2001 was the first case of glanders reported in the United States in over 50 years.[51,52] The ability of *B. mallei* to survive for up to 30 days in water at room temperature should be a consideration in development and implementation of safety, disinfection, and containment procedures for laboratories and animal facilities handling this agent.

BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of suspect cultures, animal necropsies, and for experimental animal studies. BSL-3 practices are recommended for preparatory work on cultures or contaminated materials for automated identification systems. BSL-3 practices, containment equipment, and facilities are appropriate for production operations. BSL-2 practices, containment equipment, and facilities are recommended for primary inoculation of cultures from potentially infectious clinical materials. Primary containment devices and equipment, including biological safety cabinets, safety centrifuge cups, or sealed rotors are recommended for activities likely to generate potentially infectious aerosols.

Special Issues

Be advised of possible misidentification using automated systems. For identification using MALDI-TOF MS, it is recommended to use alternative tube extraction that kills viable organisms and not direct spotting of plates in the open laboratory.

Vaccines Vaccine research and development has been conducted, but there is no available vaccine.[53]

Select Agent *B. mallei* 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 requires 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.

Burkholderia pseudomallei

Burkholderia pseudomallei is a motile, Gram-negative, oxidase-positive rod that is found in soil and water environments of equatorial regions, including Southeast Asia, Northern Australia, Madagascar, Africa, India, China, Taiwan, Central America, and South America.[54] This organism, the causative agent of melioidosis, is capable of infecting both humans and animals. A recent study estimates the global incidence of melioidosis is 165,000 cases with 89,000 deaths.[55]

Occupational Infections

Melioidosis is a disease associated with activities that expose people to soil and water such as rice farming or gardening; however, *B. pseudomallei* can be hazardous for laboratory workers, with two possible cases of aerosol transmission of melioidosis in laboratory staff.[56–58]

Natural Modes of Infection

Natural modes of transmission usually occur through direct contact with an environmental source (usually water or soil) by ingestion, percutaneous inoculation, or inhalation of the organism. In endemic areas, a significant number of agricultural workers have positive antibody titers to *B. pseudomallei* in the absence of overt disease.[59] Manifestations include localized disease, pulmonary disease, bacteremia, and disseminated disease. Abscesses can be seen in a variety of tissues and organs. However, the majority of persons exposed to this organism do not develop clinical infection.[54] Latent infection with subsequent reactivation is well recognized. Risk factors for contracting melioidosis include diabetes, liver or renal disease, chronic lung disease, thalassemia, malignancy, and immunosuppression.[54,60,61]

Laboratory Safety and Containment Recommendations

B. pseudomallei can cause systemic disease in human patients. Infected tissues and purulent drainage from cutaneous or tissue abscesses can be sources of infection as can blood and sputum. The ability of *B. pseudomallei* to survive for years in water (as well as soil) should be a consideration in development and implementation of safety, disinfection, and containment procedures for laboratories and animal facilities handling this agent.[62,63]

BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of suspect cultures, animal necropsies, and for experimental animal studies. BSL-3 practices are recommended for preparatory work on cultures or contaminated materials for automated identification systems. BSL-3 practices, containment equipment, and facilities are appropriate for production operations. BSL-2 practices, containment equipment, and facilities are recommended for primary inoculation of cultures from potentially infectious clinical materials.

Special Issues

Be advised of possible misidentification using automated systems. For identification using MALDI-TOF MS, it is recommended to use alternative tube extraction that kills viable organisms and not direct spotting of plates in the open laboratory.

Select Agent *B. pseudomallei* is a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer.[64] See Appendix F for additional information.

Transfer of Agent Importation of this agent requires 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.

Campylobacter species

Campylobacters are curved, S-shaped, or spiral Gram-negative rods associated with gastrointestinal infections, bacteremia, and sepsis. Organisms are isolated from stool specimens using selective media, reduced oxygen tension, and elevated incubation temperature (43°C) for some species, or they may be detected by molecular testing of primary clinical specimens.

Occupational Infections

These organisms rarely cause Laboratory-associated infections (LAI), although laboratory-associated cases have been documented.[65–67] Infected animals are also a potential source of infection.[68]

Natural Modes of Infection

Numerous domestic and wild animals, including poultry, pets, farm animals, laboratory animals, and wild birds, are known reservoirs and are a potential source of infection for laboratory and animal care personnel. While the infective dose is not firmly established, ingestion of as few as 350–800 organisms has caused symptomatic infection.[69–71] Natural transmission usually occurs from ingestion of organisms in contaminated food such as poultry and milk products, contaminated water, or from direct contact with infected pets and farm animals—particularly exposure to cow manure.[72] Person-to-person transmission has been documented.[73] Although the illness is usually self-limiting, relapses can occur in untreated cases and in association with some immunocompromised conditions.[74] Although infection can be mild, significant complications can occur in pregnant women, including septic abortion.[75,76]

Laboratory Safety and Containment Recommendations

Pathogenic *Campylobacter* spp. may occur in fecal specimens in large numbers. *C. fetus* subsp. fetus may also be present in blood, exudates from abscesses,

tissues, and sputa. *Campylobacter* spp. can survive for many weeks in water at 4°C. The primary laboratory hazards are ingestion and parenteral inoculation of the organism. The significance of aerosol exposure is not known.

BSL-2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Chlamydia psittaci, C. trachomatis, C. pneumoniae

Chlamydia psittaci, C. pneumoniae, and *C. trachomatis* are the three species of *Chlamydia* known to infect humans. Alternative nomenclature may include the names *Chlamydophila pneumoniae* and *Chlamydophila psittaci*. Chlamydiae are non-motile, bacterial pathogens with obligate intracellular life cycles. These three species of *Chlamydia* vary in host spectrum, pathogenicity, and in the clinical spectrum of disease. *C. psittaci* is a zoonotic agent that commonly infects psittacine (i.e., parrot family) birds and is highly pathogenic for humans. With appropriate treatment, the mortality rate for *C. psittaci* is about 1%.[77–79] *C. trachomatis* is historically considered an exclusively human pathogen. *C. pneumoniae* is considered the least pathogenic species, often resulting in subclinical or asymptomatic infections in both animals and humans. Chlamydiae have a biphasic life cycle: elementary bodies form the extracellular stage and are infective, while the reticulate bodies are intracellular and replicate by binary fission in vacuoles.[78–80]

Occupational Infections

Chlamydial infections caused by *C. psittaci* and *C. trachomatis* lymphogranuloma venereum (LGV) strains were at one time among the commonly reported laboratory-associated bacterial infections.[36,83] In cases reported before 1955, the majority of infections were psittacosis, and these had the highest case fatality rate of laboratory-associated infectious agents.[84] The major sources of laboratory-associated psittacosis are contact with and exposure to infectious aerosols in the handling, care, or the necropsy of naturally or experimentally infected birds. Infected mice and eggs also are important sources of *C. psittaci*. Most reports of Laboratory-associated infections with *C. trachomatis* attribute the infection to inhalation of large quantities of aerosolized organisms during purification or sonification procedures. Early reports commonly attributed infections to exposure

to aerosols formed during nasal inoculation of mice or inoculation of egg yolk sacs and harvest of chlamydial elementary bodies. Infections are associated with fever, chills, malaise, and headache; a dry cough is also associated with *C. psittaci* infection. Some workers exposed to *C. trachomatis* have developed conditions including mediastinal and supraclavicular lymphadenitis, pneumonitis, conjunctivitis, and keratitis.[81,85] Seroconversion to chlamydial antigens is common and often striking; however, early antibiotic treatment may prevent an antibody response. Antibiotics are effective against chlamydial infections. A case of Laboratory-associated infection attributed to inhalation of droplet aerosols with *C. pneumoniae* has been reported.[86] There has been a report of an outbreak attributed to exposure to equine fetal membranes.[87,88] With all species of Chlamydia, occupational exposures that can lead to infection most often occur through exposure to mucosal tissues in the eyes, nose, and respiratory tract.

Natural Modes of Infection

C. psittaci is the cause of psittacosis, a respiratory infection that can lead to severe pneumonia requiring intensive care support and possible death. Sequelae include endocarditis, hepatitis, abortion, and neurological complications.[78] Natural infections are acquired by inhaling dried secretions from infected birds. Psittacine birds commonly kept as pets (e.g., parrots, parakeets, cockatiels) and poultry are most frequently involved in transmission. *C. trachomatis* can cause a spectrum of clinical manifestations including genital tract infections, inclusion conjunctivitis, trachoma, pneumonia in infants, and LGV. The LGV strains cause more severe and systemic disease than do genital strains. *C. trachomatis* genital tract infections are sexually transmitted and ocular infections (trachoma) are transmitted by exposure to secretions from infected persons through contact or fomite transmission. *C. pneumoniae* is a common cause of respiratory infection; up to 50% of adults have serologic evidence of previous exposure. Infections with *C. pneumoniae* are transmitted by droplet aerosolization and are most often mild or asymptomatic, although there is research on the possible association of this agent with chronic diseases such as atherosclerosis, asthma, and others.[82,89]

Laboratory Safety and Containment Recommendations

C. psittaci may be present in the tissues, feces, nasal secretions, and blood of infected birds, and in the blood, sputum, and tissues of infected humans. *C. psittaci* can remain infectious in the environment for months and on dry, inanimate surfaces for 15 days.[90] *C. trachomatis* may be present in genital, bubo, and conjunctival fluids of infected humans. Exposure to infectious aerosols and droplets, created during the handling of infected birds and tissues, are the primary hazards to laboratory personnel working with *C. psittaci*.[91,92] The primary laboratory hazards of *C. trachomatis* and *C. pneumoniae* are accidental parenteral inoculation and direct and indirect exposure of mucous membranes of the eyes, nose, and mouth to genital, bubo, or conjunctival fluids, cell culture

materials, and fluids from infected cell cultures or eggs. Infectious aerosols, including those that may be created as a result of centrifugation, also pose a risk for infection.

BSL-3 practices and containment equipment are recommended for activities involving work with cultures, specimens, or clinical isolates known to contain or be potentially infected with the LGV serovars (L1 through L3) of *C. trachomatis*. BSL-3 practices, containment equipment, and facilities are indicated for activities with high potential for droplet or aerosol production and for activities involving large quantities or concentrations of infectious materials.

BSL-3 practices, containment equipment, and facilities are also recommended for activities involving the necropsy of infected birds and the diagnostic examination of tissues or cultures known to contain or be potentially infected with *C. psittaci* strains of avian origin. Wetting the feathers of infected birds with a detergent-disinfectant prior to necropsy can appreciably reduce the risk of aerosols of infected feces and nasal secretions on the feathers and external surfaces of the bird. ABSL-3 practices, containment equipment, and facilities and respiratory protection are recommended for personnel working with naturally or experimentally infected caged birds.

Activities involving non-avian strains of *C. psittaci* may be performed in a BSL-2 facility as long as BSL-3 practices are followed. Laboratory work with the LGV serovars of *C. trachomatis* can be conducted in a BSL-2 facility as long as BSL-3 practices are followed when handling potentially infectious materials.

BSL-2 practices, containment equipment, and facilities are recommended for personnel working with clinical specimens and cultures or other materials known or suspected to contain the ocular or genital serovars of *C. trachomatis* or *C. pneumoniae*. ABSL-2 practices, containment equipment, and facilities are recommended for activities with animals that have been experimentally infected with genital serovars of *C. trachomatis* or *C. pneumoniae*.

Special Issues

C. trachomatis genital infections are reportable infectious diseases.

Vaccines There are no human vaccines against *Chlamydia* spp.

Transfer of Agent Importation of this agent requires 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.

Clostridium botulinum and neurotoxin-producing species of Clostridia

Clostridium botulinum, and rare strains of *C. baratii* and *C. butyricum*, are anaerobic, spore-forming, Gram-positive bacilli that cause botulism, a life-threatening foodborne illness. The pathogenicity of these organisms results from the production of botulinum toxin under anaerobic conditions in which *C. botulinum* spores germinate. Please refer to Botulinum neurotoxins in Section VIII-G for biosafety guidance in handling toxin preparations.

Laboratory Safety and Containment Recommendations

Neurotoxin producing Clostridia species or its toxin may be present in a variety of food products, clinical materials (serum, feces), and environmental samples (soil, surface water) handled in the laboratory.[93] In addition, bacterial cultures may produce very high levels of toxin.[94] In healthy adults, it is typically the toxin and not the organism that causes disease. Risk of laboratory exposure is primarily due to the presence of the toxin, as opposed to infection from the organism that produces the toxin. Toxin exposure may occur through ingestion, contact with non-intact skin or mucosal membranes, or inhalation. Although spore-forming, there is no known risk from spore exposure except for the potential presence of residual toxin associated with pure spore preparations. It is recommended to use laboratory safety protocols that focus on the prevention of accidental exposure to the toxin produced by these Clostridia species.

BSL-3 practices and containment are recommended for activities with a high potential for aerosol or droplet production or for those requiring routine handling of larger quantities of the organism or toxin. ABSL-2 and BSL-2 practices, containment equipment, and facilities are recommended for diagnostic studies and titration of toxin. Before the collection of specimens, it is recommended to call the designated public health laboratory regarding any case of suspected botulism for guidance on diagnosis, treatment, specimen collection, and investigation.[95] BSL-2 practices, containment equipment, and facilities are recommended for activities that involve the organism or the toxin including the handling of potentially contaminated food.[96]

Special Issues

Select Agent Neurotoxin-producing Clostridia species are Select Agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information. See the *C. botulinum* Toxin Agent Summary Statement in Section VIII-G and Appendix I for additional information.

Transfer of Agent Importation of this agent requires 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 or its toxin to another country. See Appendix C for additional information.

Clostridioides (formerly *Clostridium*) *difficile*

Clostridioides (formerly *Clostridium*) *difficile* is a Gram-positive, spore-forming, obligate anaerobic bacillus, and it is the most common cause of infectious diarrhea in hospitalized patients.[97] The incidence of infection in the United States has increased dramatically since 2000. There were a half a million cases and 29,000 deaths reported in the United States in 2011.[98] Increases in incidence have also been observed worldwide.[99] Clinical presentations range from asymptomatic colonization to mild self-limiting diarrhea to fulminant pseudomembranous colitis, toxic megacolon, and multi-organ failure, requiring emergency colectomy.[100] Because individuals may be asymptomatically colonized with toxigenic or non-toxigenic strains of *C. difficile*, testing in the clinical diagnostic laboratory may involve one of several one, two, or three-step algorithms in an attempt to optimize sensitivity and specificity. Tests include enzyme immunoassays for free toxin or glutamate dehydrogenase, toxigenic culture, and nucleic acid amplification tests for toxin.[101]

Occupational Infections

There is a report of laboratory-associated *C. difficile* infection based on a clinical laboratory survey,[102] but cases are rare.

Natural Modes of Infection

Transmission is primarily via the fecal-oral route through hand-to-hand contact. Airborne environmental dispersal is also a route of transmission.[103,104] Most infections present during or shortly after a course of antimicrobial therapy, which disrupts the intestinal microbial composition, permitting *C. difficile* colonization and toxin production. Clindamycin, other macrolides, third-generation cephalosporins, penicillins, and fluoroquinolones are frequently associated with *C. difficile* infection.[105] Between 20–35% of patients fail initial therapy, and 60% of patients with multiple prior recurrences will fail subsequent therapy. Fecal transplantation has become a successful therapeutic option for many patients.[106,107] Asymptomatic colonization in neonates and infants (<2 years) is quite common. There is concern for an increasing incidence in children beyond this age.[108] *C. difficile* virulence factors include the exotoxins TcdA and TcdB, which bind to receptors on epithelial cells. NAP1, PCR ribotype 027 is a hypervirulent strain of *Clostridioides difficile*, which also contains a binary toxin (CDT) and a deletion in the tcdC gene that affects the production of toxins.[100] It is characterized by high-level fluoroquinolone resistance, efficient sporulation, enhanced cytotoxicity, and high toxin production. There is an associated higher mortality rate, as patients are more likely to develop life-threatening complications.[109,110] Infection or asymptomatic carriage can also occur in domestic, farm, and wild animals. *C. difficile* can be recovered from retail meats.[104]

Laboratory Safety and Containment Recommendations

Infectious fecal specimens are the most common *C. difficile*-containing specimens received in the laboratory. Endospores of *C. difficile* are impervious to desiccation, temperature fluctuations, freezing, irradiation, and many antiseptic solutions, including alcohol-based gels and quaternary ammonium-based agents.[106] Spores can survive in the environment for months to years.[104] Guidelines are available for management of healthcare-associated infections due to *C. difficile* and for cleaning to reduce the spread of the organism.[111]

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infected clinical materials or cultures. ABSL-2 facilities are recommended for studies utilizing infected laboratory animals.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Clostridium tetani and Tetanus toxin

Clostridium tetani is an anaerobic, endospore-forming, Gram-positive rod found in the soil and is an intestinal tract commensal. It produces a potent neurotoxin, tetanospasmin, which causes tetanus, an acute neurologic condition characterized by painful muscular contractions. Tetanospasmin is an exceedingly potent protein toxin that consists of a heavy chain subunit that binds the toxin to receptors on neuronal cells and a light chain subunit that blocks the release of inhibitory neural transmitter molecules within the central nervous system. The incidence of tetanus in the United States has declined steadily since the introduction of tetanus toxoid vaccines in the 1940s.[112,113]

Occupational Infections

Although the risk of infection to laboratory personnel is low, there have been some incidents of laboratory personnel exposure recorded.[84,114]

Natural Modes of Infection

Contamination of wounds by soil is the usual mechanism of transmission for tetanus. Of the 233 cases of tetanus reported to CDC from 1998 through 2000, acute injury (puncture, laceration, abrasion) was the most frequent predisposing condition. Elevated incidence rates also were observed for persons aged over 60 years, diabetics, and intravenous drug users.[112,113] When introduced into a suitable anaerobic or microaerophilic environment, *C. tetani* spores germinate

and produce tetanospasmin. The incubation period ranges from three to 21 days. The observed symptoms are primarily associated with the presence of the toxin. Wound cultures are not generally useful for diagnosing tetanus.[95,115] Tetanus is a medical emergency and immediate treatment with human tetanus immune globulin is indicated.[113]

Laboratory Safety and Containment Recommendations

The organism may be found in soil, intestinal, or fecal samples. Accidental parenteral inoculation of the toxin is the primary hazard to laboratory personnel. Because it is uncertain if tetanus toxin can be absorbed through mucous membranes, the hazards associated with aerosols and droplets remain unclear.

BSL-2 practices, containment equipment, and facilities are recommended for activities involving the manipulation of cultures or toxins. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies.

Special Issues

Vaccines It is recommended that vaccination status be considered in a risk assessment for work with this organism and/or toxin. While the risk of laboratory-associated tetanus is low, vaccination is recommended for some following risk assessment, and review of the current recommendations of the ACIP.[116]

Transfer of Agent Importation of this agent or its toxin 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.

Corynebacterium diphtheriae

Corynebacterium diphtheriae is a pleomorphic, Gram-positive rod that is isolated from the nasopharynx and skin of humans. The organism will grow on media containing 5% sheep blood, but it is recommended that primary plating include one selective agar such as cysteine-tellurite blood agar or fresh Tinsdale media incubated in 5% CO2-enriched atmosphere to separate from normal oral flora.[117] *C. diphtheriae* produces a potent exotoxin and is the causative agent of diphtheria, one of the most widespread bacterial diseases of the pre-vaccine era. The exotoxin gene is found on the beta-corynebacteriophage, which can infect non-toxigenic strains of *C. ulcerans* or *C. pseudotuberculosis*, leading to the production of toxin by these species.[118]

Occupational Infections

Laboratory-associated infections with *C. diphtheriae* have been documented.[84,119] Zoonotic infections with *C. diphtheriae* have not been recorded. *C. ulcerans* is a zoonotic pathogen that has been cultured from untreated milk and companion animals and infrequently associated with toxic infections in humans.[120,121]

Inhalation, accidental parenteral inoculation, and ingestion are the primary laboratory hazards.

Natural Modes of Infection

The agent may be present in exudates or secretions of the nose, throat (tonsil), pharynx and larynx, in wounds, blood, and on the skin. *C. diphtheriae* can be present for weeks to months in the nasopharynx and skin lesions of infected individuals and for a lifetime in asymptomatic individuals. *C. diphtheriae* can survive for up to six months on dry inanimate surfaces. Travel to endemic areas or close contact with persons who have returned recently from such areas increases risk.[122] Transmission usually occurs via direct contact with patients or carriers, and more rarely, with articles such as clothing contaminated with secretions from infected people. Naturally occurring diphtheria is characterized by the development of grayish-white, membranous lesions involving the tonsils, pharynx, larynx, or nasal mucosa. Systemic sequelae are associated with the production of diphtheria toxin, and the toxic dose of diphtheria toxin in humans is <100 ng per kg body weight.[123] An effective vaccine is available for diphtheria, and this disease has become a rarity in countries with vaccination programs.

Laboratory Safety and Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infected clinical materials or cultures. ABSL-2 facilities are recommended for studies utilizing infected laboratory animals.

Special Issues

Vaccines A licensed vaccine is available. The reader is advised to consult the current recommendations of the ACIP.[124] While the risk of laboratory-associated diphtheria is low, the administration of an adult diphtheria-tetanus toxoid at ten-year intervals may further reduce the risk of illness to laboratory and animal care personnel.[124]

Transfer of Agent Importation of this agent requires CDC and/or USDA importation permits. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Francisella tularensis

Francisella tularensis is a small, Gram-negative coccobacillus that infects numerous animal species, especially lagomorphs (including rabbits); it is the causal agent of tularemia (Rabbit fever, Deer fly fever, Ohara disease, or Francis disease) in humans. *F. tularensis* can be divided into three subspecies: *F. tularensis* (Type A), *F. holarctica* (Type B), and *F. mediasiatica*. *F. tularensis* subsp. novicida is now considered to be a separate species and referred to as

F. novicida. Type A and Type B strains are highly infectious, requiring only 10–50 organisms to cause disease, and are the main cause of tularemia worldwide.[125] The overall fatality rate of infections is <2%, but can be up to 24% for particular strains.[126] Person-to-person transmission of tularemia has not been documented. The incubation period varies with the virulence of the strain, dose, and route of introduction, but ranges from 1–14 days with most cases exhibiting symptoms in three to five days.[127] Symptoms include sudden fever, chills, headaches, diarrhea, muscle aches, joint pain, dry cough, and progressive weakness, with possible development of pneumonia. Other symptoms may include skin or mouth ulcers, swollen and painful lymph nodes, sore throat, and swollen, painful eyes.

Occupational Infections

Tularemia has been a commonly reported laboratory-associated bacterial infection.[84,128] Most cases have occurred at facilities involved in tularemia research; however, cases have been reported in diagnostic laboratories as well. Occasional cases are linked to work with naturally or experimentally infected animals or their ectoparasites.

Natural Modes of Infection

Arthropod bites (e.g., tick, deer fly, horse fly, mosquito), handling or ingesting infectious animal tissues or fluids, ingestion of contaminated water or food, and inhalation of infective aerosols are the primary transmission modes in nature. Occasionally, infections have occurred from bites or scratches by carnivores with contaminated mouthparts or claws.

Laboratory Safety and Containment Recommendations

The agent may be present in lesion exudates, respiratory secretions, CSF, blood or lymph node aspirates from patients, tissues from infected animals, fluids from infected animals, and fluids from infected arthropods. Direct contact of skin or mucous membranes with infectious materials, accidental parenteral inoculation, ingestion, and exposure to aerosols and infectious droplets have resulted in infection. Infection has been more commonly associated with cultures than with clinical materials and infected animals.[128] According to the Public Health Agency of Canada's (PHAC) Pathogen Safety Data Sheet for *F. tularensis*, the agent can survive for months to years in carcasses, organs, and straw. Additional information is available at https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/francisella-tularensis-material-safety-data-sheets-msds.html.

BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of suspect cultures, animal necropsies, and for experimental animal studies. BSL-3 practices are recommended for preparatory work prior to the use of automatic instruments that involves manipulation of

cultures. Characterized strains of reduced virulence such as LVS and SCHU S4ΔclpB can be handled with BSL-2 practices. *F. novicida* strains can also be handled with BSL-2 practices. BSL-2 practices, containment equipment, and facilities are recommended for initial activities involving clinical materials of human or animal origin suspected to contain *F. tularensis*.

Special Issues

Be advised of possible misidentification using automated systems. For identification of samples suspected of containing *F. tularensis* using MALDI-TOF MS, it is recommended to use alternative tube extraction that kills viable organisms and not direct spotting of plates in the open laboratory.

Vaccines A vaccine for tularemia is under review by the Food and Drug Administration and is not currently available in the United States.[130]

Select Agent *F. tularensis* 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 requires 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.

Helicobacter species

Helicobacter species are spiral or curved, Gram-negative rods isolated from gastrointestinal and hepatobiliary tracts of mammals and birds. There are currently 37 recognized species, including at least 14 isolated from humans. *Helicobacter pylori* is the main cause of peptic ulcer disease and a major risk factor for gastric cancer. The main habitat of *H. pylori* is the human gastric mucosa. Other *Helicobacter* spp. (*H. cinaedi*, *H. canadensis*, *H. canis*, *H. pullorum*, and *H. fennelliae*) may cause asymptomatic infection as well as proctitis, proctocolitis, enteritis and extraintestinal infections in humans.[131] Prevalence of *H. pylori* infection is decreasing worldwide, but infection is higher in certain ethnic groups and in migrants.[132]

Occupational Infections

Both experimental and accidental LAIs with *H. pylori* have been reported.[133,134] Ingestion is the primary known laboratory hazard. The importance of aerosol exposures is unknown.

Natural Modes of Infection

Chronic gastritis and duodenal ulcers are associated with *H. pylori* infection. Epidemiologic associations have also been made with gastric adenocarcinoma.[135]

Human infection with *H. pylori* may be long in duration with few or no symptoms or may present as an acute gastric illness. Transmission, while incompletely understood, is thought to be by the fecal-oral or oral-oral route.

Laboratory Safety and Containment Recommendations

H. pylori may be present in gastric and oral secretions and stool. The enterohepatic *Helicobacter* spp. (e.g., *H. canadensis*, *H. canis*, *H. cinaedi*, *H. fennelliae*, *H. pullorum*, and *H. winghamensis*) may be isolated from stool specimens, rectal swabs, and blood cultures.[131] It is recommended to incorporate processes for containment of potential aerosols or droplets into procedures involving homogenization or vortexing of gastric specimens.[136]

BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures known to contain or potentially contain the *Helicobacter* spp. ABSL-2 practices, containment equipment, and facilities are recommended for activities with experimentally or naturally infected animals.

Special Issues

Transfer of Agent Importation of this agent requires 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.

***Legionella pneumophila* and other *Legionella* spp.**

Legionella spp. are small, faintly staining, Gram-negative bacteria. They are obligately aerobic, slow-growing, nonfermentative organisms that have a unique requirement for L-cysteine and iron salts for in vitro growth. Legionellae are readily found in natural aquatic bodies and some species (*L. longbeachae*) have been recovered from soil.[137,138] They are able to colonize hot-water tanks at a temperature range from 40 to 50°C. There are currently 59 known *Legionella* species, three subspecies, and over 70 distinct serogroups of Legionella. While 30 species are known to cause human infection, the most frequent cause of human infection is *L. pneumophila* serogroup 1.[137]

Occupational Infections

Although laboratory-associated cases of legionellosis have not been reported in the literature, at least one case due to presumed aerosol or droplet exposure during animal challenge studies with *L. pneumophila* has been recorded.[139] There has been one reported case of probable human-to-human transmission of *Legionella* spp.[140]

Natural Modes of Infection

Legionella is commonly found in environmental sources, typically in man-made, warm water systems. The mode of transmission from these reservoirs is aerosolization, aspiration, or direct inoculation into the airway.[137] *Legionella* spp. may be present in amoebae from contaminated water. *Legionella* spp. have the ability to persist outside of hosts in biofilms, surviving for months in distilled water and for over a year in tap water.[141] The spectrum of illness caused by *Legionella* species ranges from a mild, self-limited, flu-like illness (Pontiac fever) to a disseminated and often fatal disease characterized by pneumonia and respiratory failure (Legionnaires' disease). Although rare, *Legionella* has been implicated in cases of sinusitis, cellulitis, pericarditis, and endocarditis.[138] Legionellosis may be either community-acquired or nosocomial. Risk factors include smoking, chronic lung disease, and immunosuppression. Surgery, especially involving transplantation, has been implicated as a risk factor for nosocomial transmission.

Laboratory Safety and Containment Recommendations

The agent may be present in respiratory tract specimens (i.e., sputum, pleural fluid, bronchoscopy specimens, lung tissue) and in extrapulmonary sites. A potential hazard may exist for the generation of aerosols containing high concentrations of the agent.

For activities likely to produce extensive aerosols or when large quantities of *Legionella* spp. are manipulated, BSL-2 with BSL-3 practices are recommended. BSL-2 practices, containment equipment, and facilities are recommended for all activities involving materials or cultures suspected or known to contain *Legionella* spp.

ABSL-2 practices, containment equipment, and facilities are recommended for activities with experimentally-infected animals. Routine processing of environmental water samples for *Legionella* may be performed with standard BSL-2 practices.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Leptospira

The genus *Leptospira* is composed of spiral-shaped bacteria with hooked ends. Leptospires are ubiquitous in nature; they are either free-living in freshwater or associated with renal infection in animals. Historically, these organisms have been classified into pathogenic (*L. interrogans*) and saprophytic (*L. biflexa*)

groups, but recent studies have identified more than 21 species based on genetic analysis, nine of which are definitive pathogens.[142] These organisms also have been characterized serologically, with more than 200 pathogenic and 60 saprophytic serovars identified.[142] These organisms are the cause of leptospirosis, a zoonotic disease of worldwide distribution. Growth of leptospires in the laboratory requires specialized media and culture techniques, and cases of leptospirosis are usually diagnosed by serology.

Occupational Infections

Leptospirosis is a well-documented, laboratory hazard. In older literature, 70 LAIs and ten deaths have been reported.[36,84] Direct and indirect contact with fluids and tissues of experimentally or naturally infected mammals during handling, care, or necropsy are potential sources of infection.[143,144] A laboratory-associated case caused by percutaneous exposure to broth cultures of *Leptospira* was reported in 2004.[145] It is important to remember that rodents are natural carriers of leptospires. Animals with chronic renal infection shed large numbers of leptospires in the urine continuously or intermittently for long periods of time. *Leptospira* spp. may persist for weeks in soil contaminated with infected urine. Rarely, infection may be transmitted by bites of infected animals.[143]

Natural Modes of Infection

Human leptospirosis typically results from direct contact with infected animals, contaminated animal products, or contaminated water sources. Common routes of infection are abrasions, cuts in the skin or via the conjunctiva. Higher rates of infection are observed in agricultural workers and workers in other occupations associated with animal contact. Human-to-human transmission is rare. Leptospirosis can cause the following symptoms: fever, headache, chills, muscle aches, vomiting, jaundice, red eyes, abdominal pain, diarrhea, and rash. After an initial phase of illness, the patient may recover, then become ill again with another more severe phase that can involve kidney failure, liver failure, or meningitis (Weil's Disease).[146]

Laboratory Safety and Containment Recommendations

The organism may be present in urine, blood, and tissues of infected animals and humans. Asymptomatic infection may occur in carrier animals and humans. Ingestion, parenteral inoculation, and direct and indirect contact of skin or mucous membranes, particularly the conjunctiva, with cultures or infected tissues or body fluids are the primary laboratory hazards. The importance of aerosol exposure is unclear, but occasional cases of inhalation of droplets of urine or water have been suspected.[147]

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infective

tissues, body fluids, and cultures. ABSL-2 practices are recommended for the housing and manipulation of infected animals.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Listeria monocytogenes

Listeria monocytogenes is a Gram-positive, catalase-positive, non-spore forming, aerobic bacillus that is weakly beta-hemolytic on sheep blood agar.[148] The organism has been isolated from soil, animal feed (silage), and a wide range of human foods and food processing environments. It may also be isolated from symptomatic/asymptomatic animals (particularly ruminants) and humans.[149] This organism is the causative agent of listeriosis, a foodborne disease of humans and animals.

Occupational Infections

Cutaneous listeriosis, characterized by pustular or papular lesions on the arms and hands, has been described in veterinarians and farmers.[150] Asymptomatic carriage has been reported in laboratorians.[151]

Natural Modes of Infection

Most human cases of listeriosis result from eating contaminated foods, notably soft cheeses, ready-to-eat meat products (e.g., hot dogs, luncheon meats), pâté, and smoked fish/seafood.[149] Listeriosis can present in healthy adults with symptoms of fever and gastroenteritis; pregnant women and their fetuses; newborns; and persons with impaired immune function are at greatest risk of developing severe infections including sepsis, meningitis, and fetal demise. In pregnant women, *L. monocytogents* infections occur most often in the third trimester and may precipitate labor. Transplacental transmission of *L. monocytogenes* poses a grave risk to the fetus.[152]

Laboratory Safety and Containment Recommendations

Listeria monocytogenes may be found in feces, CSF, and blood, as well as numerous food and environmental samples.[149] *L. monocytogenes* is somewhat heat-resistant, can tolerate (and replicate in) cold temperatures, can survive at low pH conditions, and can be resistant to some disinfectants such as quaternary ammonium compounds.[153,154] Naturally or experimentally infected animals are a source of exposure to laboratory workers, animal care personnel, and other animals. While ingestion is the most common route of exposure, Listeria can also cause eye and skin infections following direct contact with the organism.

BSL-2 practices, containment equipment, and facilities are recommended when working with clinical specimens and cultures known or suspected to contain Listeria. ABSL-2 practices, containment equipment, and facilities are recommended for activities involving experimentally or naturally infected animals. Due to potential risks to the fetus, it is recommended that pregnant women be advised of the risk of exposure to *L. monocytogenes*.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Mycobacterium leprae

Mycobacterium leprae is a Gram-positive bacterium and is the causative agent of leprosy, also called Hansen's disease. *M. leprae* are intracellular bacteria that cannot be cultured using laboratory medium. Bacteria can be recovered from infected tissues and propagated in laboratory animals, specifically the nine-banded armadillo. *M. lepromatosis* are related bacteria that have now been identified to cause similar disease.[155]

Occupational Infections

There are no cases of occupational acquisition of *M. leprae* reported as a result of working in a laboratory or being in contact with clinical materials of human or animal origin.

Natural Modes of Infection

Leprosy is transmitted from person-to-person following prolonged exposure, presumably via contact with respiratory secretions from infected individuals or animals. Naturally-occurring leprosy has been reported in armadillos, with both humans and armadillos recognized as reservoirs for infection.[156,157] Although transmission from armadillos to humans has not been definitively proven, it is likely since contact with armadillos is a significant risk factor for acquisition of human disease.[158,159] Cases in the United States have recently been seen in Texas, Florida, and Louisiana.[160,161] Endemic animal forms of the disease have been described due to related organisms.[162]

Laboratory Safety and Containment Recommendations

M. leprae may be present in tissues and exudates from lesions of infected humans and experimentally or naturally infected animals. Direct contact of the skin and mucous membranes with infectious materials and parenteral inoculation are the primary potential laboratory hazards associated with handling infectious clinical materials.

Selection of an appropriate disinfectant is an important consideration for laboratories working with mycobacteria. See Appendix B for additional information.

BSL-2 practices, containment equipment, and facilities are recommended for all activities with known or potentially infectious materials from humans and animals. It is recommended to use extraordinary care to avoid accidental parenteral inoculation with contaminated sharp instruments. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies utilizing rodents, armadillos, and NHPs.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Mycobacterium tuberculosis complex

The *Mycobacterium tuberculosis* complex includes the species *M. tuberculosis*, *M. bovis, M. africanum, M. caprae, M. microti, M. canettii, M. pinnipedii*, and the recently described species *M. mungi* and *M. orygis*.[163,164] *M. tuberculosis* grows slowly, typically requiring several weeks for formation of colonies on solid media. Incubation in broth culture can at times reduce the incubation time to less than one week if the inoculum is sufficient.[163] The organism has a thick, lipid-rich cell wall that renders bacilli resistant to harsh treatments including alkali and detergents. Mycolic acid in the cell wall results in a positive acid-fast stain.

Occupational Infections

M. tuberculosis and *M. bovis* infections are a proven hazard to laboratory personnel and others who may be exposed to infectious aerosols in the laboratory, autopsy rooms, and other healthcare facilities.[36,84,165–169] The incidence of tuberculosis in health care personnel working with *M. tuberculosis*-infected patients has been reported to be significantly higher than that of those not working with the agent.[170] Multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains are of particular concern.[109,171] Naturally or experimentally infected NHPs are a proven source of human infection.[172] Experimentally-infected guinea pigs and mice do not pose the same hazard because droplet nuclei are not produced by coughing in these species; however, litter from infected animal cages may become contaminated and serve as a source of infectious aerosols.

Natural Modes of Infection

M. tuberculosis is the etiologic agent of tuberculosis, a leading cause of morbidity and mortality worldwide. Infectious aerosols produced by coughing spread disease from person to person. Some individuals will develop active disease

within months of infection, and some of those will clear the infection completely. Others will achieve immunological control with latent (but viable) organisms, with potential for reactivation later upon immunosuppression. Approximately 5–10% of latent infections progress to active infections. The primary focus of infection is the lungs, but extra-pulmonary disease does occur, primarily in immunocompromised individuals. Miliary (disseminated) tuberculosis has the most serious consequences with meningitis developing in 50% of cases, along with a high fatality rate if not treated effectively. HIV infection is a serious risk factor for the development of active disease. *M. bovis* is primarily found in animals but can also infect humans. It is spread to humans, primarily children, by consumption of non-pasteurized milk and dairy products, by handling of infected carcasses, or by inhalation. Human-to-human transmission of *M. bovis* via aerosols is possible.

Laboratory Safety and Containment Recommendations

Tubercle bacilli may be present in sputum, gastric lavage fluids, CSF, urine, and in a variety of tissues. Exposure to laboratory-generated aerosols is the most important laboratory hazard encountered. Tubercle bacilli may survive in heat-fixed smears and, if present, may be aerosolized in the preparation of frozen tissue sections.[171] Because of the low infective dose of *M. tuberculosis* (<10 bacilli), it is recommended that sputa and other clinical specimens from suspected or known cases of tuberculosis be considered potentially infectious and handled with appropriate precautions. Mycobacteria can be resistant to disinfection and may survive on inanimate surfaces for long periods. Needlesticks are also a recognized hazard. Selection of an appropriate disinfectant is an important consideration for laboratories working with mycobacteria. See Appendix B for additional information.

BSL-3 practices, containment equipment, and facilities are recommended for laboratory activities in the propagation and manipulation of cultures of any of the subspecies of the *M. tuberculosis* complex. Use of a slide-warming tray, rather than a flame, is recommended for fixation of slides. ABSL-3 practices are recommended for animal studies using experimentally or naturally infected NHPs or immunocompromised mice, as high titers may be found in organs from immunocompromised animals. Animal studies using rodents (e.g., guinea pigs, rats, rabbits, mice) can be conducted at ABSL-2 with ABSL-3 practices.[174] All airborne infections of rodents using *M. tuberculosis* must be performed in an appropriate ABSL-3 laboratory.

BSL-2 practices and procedures, containment equipment, and facilities are recommended for non-aerosol-producing manipulations of clinical specimens. Manipulation of small quantities of the attenuated vaccine strain *M. bovis* Bacillus Calmette-Guérin (BCG) can be performed at BSL-2 in laboratories that do not

culture *M. tuberculosis* and do not have BSL-3 facilities. However, considerable care is suggested to verify the identity of the strain and to ensure that cultures are not contaminated with virulent *M. tuberculosis* or other *M. bovis* strains.

Special Issues

Be advised of possible misidentification using automated systems. For identification using MALDI-TOF MS, it is recommended to use alternative tube extraction that kills viable organisms in the BSC, and not direct spotting of plates in the open laboratory.

Surveillance Annual or semi-annual skin testing with purified protein derivative (PPD) or FDA-approved Interferon-Gamma Release Assay (IGRA) of previously skin-test-negative personnel can be used as a surveillance procedure.[175]

Vaccines The attenuated live BCG is available and used in other countries but is not generally recommended for use in the United States.

Transfer of Agent Importation of this agent requires 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.

Mycobacterium spp. other than *M. tuberculosis* complex and *M. leprae*

There are over 150 *Mycobacterium* species including both slowly and rapidly growing species.[163] In the past, mycobacterial isolates that were not identified as *M. tuberculosis* complex were often called atypical mycobacteria, but these are now more commonly referred to as nontuberculous mycobacteria (NTM) or mycobacteria other than tuberculosis (MOTT). The majority of mycobacterial species are common environmental organisms. There has been a perceived increase in NTM isolated from hospitalized patients over the past 20 years.[176,177] Approximately 25 species are associated with human infections, with a number of additional species associated with infections in immunocompromised persons.[178] All of these species are considered opportunistic pathogens in humans, and they are not considered generally communicable; however, there is evidence of transmission between some individuals with chronic diseases.[179] The most common types of infections and causes are:

1. Pulmonary disease with a clinical presentation resembling tuberculosis caused by *M. kansasii, M. avium*, and *M. intracellulare*;
2. Lymphadenitis associated with *M. avium, M. scrofulaceum*, and other rapidly growing mycobacteria;[180]
3. Disseminated infections in immunocompromised individuals caused by *M. avi*um and *M. intracellulare*;

4. Pulmonary infection or colonization of patients with cystic fibrosis caused by *M. avium* complex, *M. kansasii, M. abscessus*, and other rapidly growing mycobacteria;[181,182] and
5. Skin ulcers and soft tissue wound infections including Buruli ulcer caused by *M. ulcerans*, granulomas caused by *M. marinum* associated with exposure to organisms in freshwater and saltwater and fish tanks, and tissue infections resulting from trauma or surgical procedures caused by *M. fortuitum, M. chelonae*, and *M. abscessus*.

Occupational Infections

A Laboratory-associated infection with *Mycobacterium* spp. other than *M. tuberculosis* complex was reported when a laboratory worker injected bacteria into his thumb while performing experiments on mice.[183]

Natural Modes of Infection

Person-to-person transmission is not considered common, but there is evidence for transmission in some populations.[179] Presumably, pulmonary infections are most often the result of inhalation of aerosolized bacilli, most likely from the surface of contaminated water. Mycobacteria are widely distributed in the environment and in animals, and zoonoses have occurred.[184,185] They are also common in potable water supplies, perhaps as the result of the formation of biofilms.

Laboratory Safety and Containment Recommendations

Various species of mycobacteria may be present in sputa, exudates from lesions, tissues, and in environmental samples. Mycobacteria can be resistant to disinfection and survive on inanimate surfaces and for long periods in natural and tap water sources. Direct contact of skin or mucous membranes with infectious materials, ingestion, and parenteral inoculation are the primary laboratory hazards associated with clinical materials and cultures. Aerosols created during the manipulation of broth cultures or tissue homogenates of these organisms also pose a potential infection hazard.

BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures of *Mycobacterium* other than *M. tuberculosis* complex. Clinical specimens may also contain *M. tuberculosis* and laboratory workers are advised to exercise caution to ensure the correct identification of mycobacterial isolates. Special caution is recommended in handling *M. ulcerans* and *M. marinum* to avoid skin exposure. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies. Selection of an appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria. See Appendix B for additional information.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Neisseria gonorrhoeae

Neisseria gonorrhoeae is a Gram-negative, oxidase-positive diplococcus associated with gonorrhea, a sexually transmitted disease of humans. The organism may be isolated from clinical specimens and cultivated in the laboratory using specialized growth media.[186] Infection is often diagnosed using molecular methods on direct clinical specimens.

Occupational Infections

Laboratory-associated gonococcal infections have been reported in the United States and elsewhere.[187–189] These infections have presented as conjunctivitis, with either direct finger-to-eye contact or exposure to splashes of either liquid cultures or contaminated solutions proposed as the most likely means of transmission.

Natural Modes of Infection

Gonorrhea is a sexually transmitted disease of worldwide importance. The 2016 rate of reported infection for this disease in the United States was 145.8 per 100,000 population, a steady increase from a low of 98.1 infections per 100,000 population recorded in 2009.[191] The natural mode of infection is through direct contact with exudates from mucous membranes of infected individuals. This usually occurs by sexual activity, although newborns may also become infected during birth.[186]

Laboratory Safety and Containment Recommendations

The agent may be present in conjunctival, urethral and cervical exudates, synovial fluid, urine, feces, blood, and CSF. Parenteral inoculation and direct or indirect contact of mucous membranes with infectious clinical materials are known primary laboratory hazards. Laboratory-associated illness due to aerosol transmission has not been documented.

Additional primary containment and personnel precautions such as those described for BSL-3 may be indicated when there is high risk of aerosol or droplet production and for activities involving production quantities or high concentrations of infectious materials. BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of clinical materials or cultures. Animal studies may be performed at ABSL-2.

Special Issues

Neisseria gonorrhoeae has gained resistance to several classes of antimicrobials over the last few decades, making the organism increasingly difficult to treat. Fluoroquinolones, oral cephalosporins such as cefixime, and doxycycline are no longer recommended for treatment of uncomplicated gonorrhea. An extensively drug-resistant (XDR) strain has been reported and is being monitored, and currently, there are no other effective treatments for XDR gonorrhea.[192]

Transfer of Agent Importation of this agent requires 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.

Neisseria meningitidis

Neisseria meningitidis is a Gram-negative diplococcus which can cause serious invasive bacterial infections, with clinical manifestations including serious acute meningitis and septicemia in humans. Virulence is associated with the expression of a polysaccharide capsule. Among the thirteen defined *N. meningitidis* capsular serogroups, six are the main causes of invasive meningococcal disease (serogroups A, B, C, W, X and Y). The handling of *N. meningitidis* isolates, particularly from sterile body sites, and/or clinical specimens containing live *N. meningitidis* may increase the risk of transmission for microbiologists.[193]

Occupational Infections

Manipulating suspensions of *N. meningitidis* outside a BSC is associated with a high risk for contracting meningococcal disease.[193,194] Microbiologists have been shown to have a much higher infection rate compared to that of the United States' general population aged 30–59 years, and a case fatality rate of 50%—substantially higher than the 12–15% associated with disease among the general population. Almost all the microbiologists identified as having an LAI had manipulated invasive *N. meningitidis* isolates on an open laboratory bench.[195] Rigorous protection from droplets or aerosols (including the use of a BSC) is recommended when microbiological procedures are performed on all *N. meningitidis* isolates. Although there are some molecular assays that can detect *N. meningitidis* directly in clinical specimens, cultures are still routinely performed.

Natural Modes of Infection

The human upper respiratory tract is the natural reservoir for *N. meningitidis*. Invasion of organisms from the respiratory mucosa into the circulatory system causes infection that can range in severity from subclinical to fulminant fatal disease. Transmission occurs from person-to-person and is usually mediated by direct contact with respiratory droplets from infected individuals.

Laboratory Safety and Containment Recommendations

N. meningitidis may be present in pharyngeal exudates, CSF, blood, saliva, sterile body sites (most commonly CSF and blood), and in rare cases, urine or urethral (genital) discharge. Parenteral inoculation, droplet exposure of mucous membranes, infectious aerosol generation and ingestion are the primary hazards to laboratory personnel. Based on the mechanism of natural infection and the risk associated with the handling of isolates on an open laboratory bench, exposure to droplets or aerosols of *N. meningitidis* is the most likely risk for infection in the laboratory. Although *N. meningitidis* does not survive well outside of a host, the organism is able to survive on plastic and glass from hours to days at room temperature.

BSL-3 practices and procedures are indicated for activities with a high potential for droplet or aerosol production and for activities involving production quantities or high concentrations of infectious materials. BSL-2 practices, containment equipment, and facilities are recommended for handling bacterial cultures and inoculation of clinical materials. It is recommended to handle all *N. meningitidis* cultures within a BSC. ABSL-2 conditions are recommended for animal studies.

Special Issues

Vaccines For protection against *N. meningitidis* serogroups A, C, Y, and W-135, there are commercially available polysaccharide and conjugate vaccines. These are recommended to be administered to otherwise healthy children in adolescence with a booster in late adolescence.[193] Recently, a meningococcal serogroup B vaccine has become available. Both vaccines are necessary for full protection as one does not confer immunity for the other.[196] Vaccination with both vaccines is recommended for laboratorians who handle live bacteria and may be exposed to *N. meningitidis*.[193,197,198]

Transfer of Agent Importation of this agent requires 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.

Salmonella serotypes, other than *S. enterica* serotype Typhi (*S.* Typhi)

Salmonellae are Gram-negative, enteric bacteria associated with diarrheal illness in humans. They are motile oxidase-negative organisms that are easily cultivated on standard bacteriologic media, although enrichment and selective media may be required for isolation from clinical specimens. *Salmonellae* can easily be isolated using selective and differential media or may be detected by molecular testing of primary clinical specimens. Taxonomic studies have organized this genus into two species, *S. enterica* and *S. bongori*, containing more than 2,500 antigenically distinct serotypes.[199,200] *S. enterica* contains the vast majority of

serotypes associated with human disease. *S. enterica* serotypes Typhimurium and Enteritidis are the serotypes most frequently encountered in the United States. This summary statement covers all serotypes except *S*. Typhi.

Occupational Infections

Salmonellosis is a documented hazard to laboratory personnel.[114,201–204] Primary reservoir hosts include a broad-spectrum of domestic and wild animals, including birds, mammals, and reptiles, all of which may serve as a source of infection to laboratory personnel. Case reports of LAIs indicate a presentation of symptoms similar to those of naturally-acquired infections.[205]

Natural Modes of Infection

Salmonellosis is a foodborne disease of worldwide distribution. An estimated one million foodborne cases of salmonellosis occur annually in the United States, and the global burden of non-typhoidal disease is estimated to be 94 million cases and 155,000 deaths annually.[206–208] A wide range of domestic and feral animals (e.g., poultry, swine, rodents, cattle, iguanas, turtles, chicks, dogs, cats, and others) may serve as reservoirs for this disease, as well as humans.[209,210] Some human carriers shed the bacteria for years and some patients recovering from *S. enterica* infections may shed the bacteria for months. Animals can also have a latent or carrier state with long-term shedding of the bacteria. The most common mode of transmission is by ingestion of food from contaminated animals or contamination during processing. The disease usually presents as acute enterocolitis (fever, severe diarrhea, abdominal cramping), with an incubation period ranging from six to 72 hours, most often lasting four to seven days and patients tend to recover without treatment. Antimicrobial therapy is not recommended for uncomplicated *Salmonella*-related gastroenteritis.[206] Bacteremia occurs in 3–10% of individuals infected with *S. enterica*. Antimicrobial resistance of *Salmonella* spp. is becoming a problem worldwide, and this is a concern for invasive disease.[211]

Laboratory Safety and Containment Recommendations

The agent may be present in feces, blood, urine, food, feed, and environmental materials. Some *Salmonella* spp. may survive for long periods in food, feces, water, and on surfaces. Ingestion and parenteral inoculations are the primary laboratory hazards. Naturally or experimentally infected animals are a potential source of infection for laboratory and animal care personnel and for other animals.

BSL-2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures. It is recommended that special emphasis be placed on personal protective equipment, handwashing, manipulation of faucet handles, and decontamination of work surfaces to decrease the risk of LAI. For work involving production quantities or high concentrations of cultures, and for activities with a high potential for

aerosol production, it is recommended that a BSC be used and that centrifugation be performed using autoclavable, aerosol-tight rotors and safety cups. ABSL-2 facilities and practices are recommended for activities with experimentally infected animals.[199]

Special Issues

Vaccines Human vaccines against non-typhoidal strains are not available.[212]

Transfer of Agent Importation of this agent requires 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.

Salmonella enterica serotype Typhi (*S.* Typhi)

The genus *Salmonella* is divided into two species, *S. enterica* and *S. bongori*, containing more than 2,500 antigenically distinct subtypes or serotypes.[200] *S. enterica* contains the vast majority of serotypes associated with human disease. *S. enterica* serotype Typhi, commonly designated *S*. Typhi, is the causative agent of typhoid fever. Untreated case mortality for typhoid fever is >10%.[213] *S*. Typhi is a motile, Gram-negative, enteric bacterium that is easily cultivated on standard bacteriologic media, although enrichment and selective media may be required for isolation of this organism from clinical materials. *S*. Typhi can easily be isolated using selective and differential media, or it may be detected by molecular testing of primary clinical specimens. *S. enterica* serotype Paratyphi (*S.* Paratyphi) is also considered a typhoidal serovar causing a similar illness.

Occupational Infections

Typhoid fever is a demonstrated hazard to laboratory personnel and students working with *S*. Typhi in teaching laboratories with many Laboratory-associated infections and several resulting fatalities being reported.[84,114,203] Ingestion and, less frequently, parenteral inoculation are the most significant modes of transmission in the laboratory. Secondary transmission to other individuals outside of the laboratory is also a concern. Laboratory-associated *S*. Typhi infections usually present with headache, abdominal pain, high fever, and possible septicemia.[203]

Natural Modes of Infection

Typhoid fever is a serious, potentially lethal, bloodstream infection associated with sustained high fever and headaches. It is common in the developing world with 25 million infections and >200,000 deaths annually but rare in the United States with only 400 cases annually.[214–216] Less than 1% of cases in the U.S. are lethal, and these cases are often associated with foreign travel. Humans are the sole reservoir, and asymptomatic carriers may occur. The infectious dose is low

(<1000 organisms), and the incubation period may vary from one to six weeks depending upon the dose of the organism. The natural mode of transmission is by ingestion of food or water contaminated by feces or urine of patients or asymptomatic carriers.[199,206] Antimicrobial resistance of *S.* Typhi is a significant global concern.[217]

Laboratory Safety and Containment Recommendations

The agent may be present in feces, blood, bile, and urine. Humans are the only known natural reservoir of infection. Ingestion and parenteral inoculation of the organism represent the primary laboratory hazards. The importance of aerosol exposure in previous cases is not known. To avoid possible secondary transmission related to contaminated surfaces and clothing in teaching laboratories, the use of nonpathogenic strains is recommended.

BSL-3 practices and equipment are recommended for activities likely to produce significant aerosols or for activities involving production quantities of organisms. BSL-2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures. It is recommended that special emphasis be placed on personal protective equipment, handwashing, manipulation of faucet handles, and decontamination of work surfaces to decrease the risk of LAI.

It is recommended that centrifugation be performed using autoclavable aerosol-tight rotors or safety cups. ABSL-2 facilities, practices, and equipment are recommended for activities with experimentally infected animals.

Special Issues

Vaccines Vaccines for *S*. Typhi are available and it is recommended that personnel regularly working with potentially infectious materials consider vaccination. The reader is advised to consult the current recommendations of the Advisory Committee on Immunization Practices (ACIP).[218]

Transfer of Agent Importation of this agent requires 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.

Shiga toxin (Verocytotoxin)-producing *Escherichia coli*

Escherichia coli (*E. coli*) is one of six species in the Gram-negative genus *Escherichia*. This organism is a common inhabitant of the bowel flora of healthy humans and other mammals and is one of the most extensively studied prokaryotes. An extensive serotyping system has been developed for *E. coli* based on the O (somatic) and H (flagellar) antigens expressed by these organisms. Certain

pathogenic clones of *E. coli* may cause urinary tract infections, bacteremia, meningitis, and diarrheal disease in humans, and these clones are associated with specific serotypes.[199]

The diarrheagenic *E. coli* strains have been characterized into at least five basic pathogenicity groups: Shiga toxin (Verocytotoxin)-producing *E. coli* (a subset are referred to as enterohemorrhagic *E. coli*), enterotoxigenic *E. coli*, enteropathogenic *E. coli*, enteroinvasive E. coli, and enteroaggregative *E. coli*.[199] In addition to clinical significance, *E. coli* strains are routinely used as hosts for cloning experiments and other genetic manipulations in the laboratory. This summary statement only provides recommendations for safe manipulation of Shiga toxin-producing *E. coli* strains.

Occupational Infections

Shiga toxin-producing *E. coli* strains, including strains of serotype O157:H7, are a demonstrated hazard to laboratory personnel with the majority of reported Laboratory-associated infections being caused by enterohemorrhagic *E. coli*.[219–223] Sources of infection include ingestion from contaminated hands and contact with infected animals. The infectious dose is estimated to be low, similar to that reported for *Shigella* spp., at 10–100 organisms.[223]

Natural Modes of Infection

Cattle represent the most common natural reservoir of Shiga toxin-producing *E. coli*, but it has also been detected in wild birds and rodents in close proximity to farms.[224] Transmission usually occurs by ingestion of contaminated food, including raw milk, fruits, vegetables, and particularly ground beef. Human-to-human transmission has been observed in families, daycare centers, and custodial institutions. Waterborne transmission has been reported from outbreaks associated with swimming in a crowded lake and drinking unchlorinated municipal water.[225–227] *E. coli* has the ability to survive from hours to months on inanimate surfaces. In a small number of patients (usually children) infected with these organisms, the disease progresses to hemolytic uremic syndrome or death.

Laboratory Safety and Containment Recommendations

Shiga toxin-producing *E. coli* are usually isolated from feces. However, a variety of food specimens contaminated with the organisms including uncooked ground beef, unpasteurized dairy products, and contaminated produce may present laboratory hazards. This agent may also be found in blood or urine specimens from infected humans or animals. Ingestion is the primary laboratory hazard. The importance of aerosol exposure is not known.

BSL-2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures.

It is recommended that special emphasis be placed on personal protective equipment, handwashing, manipulation of faucet handles, and decontamination of work surfaces to decrease the risk of LAI. For work involving production quantities or high concentrations of cultures, and for activities with a high potential for aerosol production, it is recommended that a BSC be used and that centrifugation be performed using autoclavable aerosol-tight rotors and safety cups. ABSL-2 facilities and practices are recommended for activities with experimentally infected animals.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Shigella

The genus *Shigella* is composed of non-motile, Gram-negative bacteria in the family *Enterobacteriaceae*. There are four subgroups that have been historically treated as separate species including: subgroup A (*Shigella dysenteriae*), subgroup B (*S. flexneri*), subgroup C (*S. boydii*), and subgroup D (*S. sonnei*). Members of the genus *Shigella* have been recognized since the late 19th century as causative agents of bacillary dysentery, or shigellosis.[199] Shigella can easily be isolated using selective and differential media, or it may be detected by molecular testing of primary clinical specimens.

Occupational Infections

Shigellosis is one of the most frequently reported Laboratory-associated infections in the United States.[102,114] A survey of 397 laboratories in the United Kingdom revealed that in 1994–1995, four of nine reported Laboratory-associated infections were caused by *Shigella*.[228] The direct handling of isolates and animal work, such as experimentally infecting guinea pigs, other rodents, and NHPs are proven sources of Laboratory-associated infection.[114,229]

Natural Modes of Infection

Humans and other large primates are the only natural reservoirs of *Shigella* bacteria. Most transmission is by the fecal-oral route; infection also is caused by ingestion of contaminated food or water.[199] Infection with *Shigella dysenteriae* type 1 causes more severe, prolonged, and frequently fatal illness than does infection with other *Shigella* spp., with a fatality rate up to 20%. Complications of shigellosis can include hemolytic uremic syndrome and reactive arthritis (Reiter's syndrome).[230]

Laboratory Safety and Containment Recommendations

The agent may be present in feces and, rarely, in the blood of infected humans or animals. The organism can be shed for weeks after infection and it is communicable as long as the organism is present in the feces. *Shigella* spp. can survive for days in feces and water. Ingestion is the primary laboratory hazard and to a lesser extent, parenteral inoculation of the agent and person-to-person transmission are potential laboratory hazards. Although rare, experimentally-infected guinea pigs and other rodents can transmit infection to laboratory staff. The 50% infectious dose (oral) of Shigella for humans is only 180 organisms.[114] The importance of aerosol exposure is not known.

BSL-2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures. It is recommended that special emphasis be placed on personal protective equipment, handwashing, manipulation of faucet handles, and decontamination of work surfaces to decrease the risk of LAI. For work involving production quantities or high concentrations of cultures, and for activities with a high potential for aerosol production, it is recommended that a BSC be used and that centrifugation be performed using autoclavable, aerosol-tight rotors and safety cups. ABSL-2 facilities and practices are recommended for activities with experimentally-infected animals.

Special Issues

Vaccines Vaccines are currently not available for use in humans.

Transfer of Agent Importation of this agent requires 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.

Staphylococcus aureus **(Methicillin-Resistant, Vancomycin-Resistant, or Vancomycin-Intermediate)**

Staphylococcus aureus is a Gram-positive bacterium associated with a wide spectrum of diseases in humans, ranging from minor to severe. *S. aureus* is a catalase-positive coccus that is a non-motile, non-spore forming facultative anaerobe. *S. aureus* isolates express a coagulase factor, which differentiates them from other staphylococci that colonize humans. *S. aureus* is easily cultivated on standard and selective media, such as high mannitol salt agar. Several molecular tests are also available for testing from clinical specimens. Methicillin-resistant *S. aureus* (MRSA) is common in most areas of the world, with a resistance rate of 30% in most of North America. Vancomycin is currently the treatment of choice for MRSA.[231] Vancomycin-resistant *S. aureus* (VRSA) (vancomycin MIC ≥ 16 µg/mL) is rare, with only 14 cases documented in the

United States, in addition to unconfirmed cases in India and Iran.[232] Vancomycin-intermediate *S. aureus* (VISA) (i.e., isolates with reduced susceptibility to vancomycin, defined as a MIC of 4–8 µg/mL) have been documented at a higher rate, but remain uncommon in most hospitals.[233] To date, all isolates of VRSA and VISA have remained susceptible to other FDA-approved drugs.

Occupational Infections

Several cases of laboratory-associated MRSA infections have been documented.[234–236] To date, no laboratory or occupational infections due to VISA or VRSA have been reported. Case reports of Laboratory-associated infections include nasal colonization and minor skin infections. Guidelines have been provided for investigation and control of VRSA in healthcare settings.[235]

Natural Modes of Infection

S. aureus (including MRSA and VISA) is part of the normal human flora, found primarily in the nares and on the skin of primarily the groin and axillae. Approximately 20% of the population is persistently colonized by *S. aureus*, and 60% are colonized intermittently.[238] Animals may act as reservoirs, including livestock and companion animals.[239] *S. aureus* is an opportunistic pathogen that causes a wide variety of diseases in humans. The organism is a leading cause of foodborne gastroenteritis, as a result of consumption of food contaminated with enterotoxins expressed by some strains. Skin conditions caused by *S. aureus* include cellulitis, scalded skin syndrome, furuncles, carbuncles, impetigo, and abscesses. Certain strains of *S. aureus* express toxic shock syndrome toxin-1 (TSST-1), which is responsible for toxic shock syndrome. *S. aureus* is also a common cause of surgical site infections, endocarditis, peritonitis, pneumonia, bacteremia, meningitis, osteomyelitis, and septic arthritis. Infection modes include ingestion of food containing enterotoxins and person-to-person transmission via contact with colonized health care workers to patients. Nasal colonization can lead to auto-infection.

Laboratory Safety and Containment Recommendations

The agent may be present in many human specimens and in food. Primary hazards to laboratory personnel are direct and indirect contact of broken skin or mucous membranes with cultures and contaminated laboratory surfaces, parenteral inoculation, and ingestion of contaminated materials.

BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infected clinical materials or cultures. ABSL-2 facilities are recommended for studies utilizing infected laboratory animals.

Special Issues

Vaccines Vaccines are currently not available for use in humans.

Transfer of Agent Importation of this agent requires 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.

Treponema pallidum

Treponema pallidum is a species of extremely fastidious spirochetes that die readily upon desiccation or exposure to atmospheric levels of oxygen and have not been cultured continuously in vitro.[240] *T. pallidum* cells have lipid-rich outer membranes and are highly susceptible to disinfection with common alcohols (i.e., 70% isopropanol). This species contains three subspecies including *T. pallidum* subsp. pallidum (associated with venereal syphilis), *T. pallidum* subsp. *endemicum* (associated with endemic syphilis), and *T. pallidum* subsp. *pertenue* (associated with yaws). These organisms are obligate human pathogens.

Occupational Infections

T. pallidum is a documented hazard to laboratory personnel, but there have been no reported cases since the 1970s.[84,241] Experimentally-infected animals are a potential source of infection. Syphilis has been transmitted to personnel working with a concentrated suspension of *T. pallidum* obtained from an experimental rabbit orchitis.[242] Rabbit-adapted *T. pallidum* (Nichols strain and possibly others) retains virulence for humans, and rabbits are used in both clinical and research laboratories to isolate clinical strains and model venereal syphilis, respectively.[243] A murine model was recently developed to study venereal syphilis.[244]

Natural Modes of Infection

Humans are the only known natural reservoir of *T. pallidum*; though, non-human primates may be a potential reservoir.[245] Transmission occurs via direct sexual contact (venereal syphilis), direct skin contact (yaws), or direct mucous membrane contact (endemic syphilis). Venereal syphilis is a sexually transmitted disease that occurs worldwide, whereas yaws occurs in tropical areas of Africa, South America, the Caribbean, and Indonesia. Endemic syphilis is limited to arid areas of Africa and the Middle East.[246]

Laboratory Safety and Containment Recommendations

The agent may be present in materials collected from cutaneous and mucosal lesions and in blood. *T. pallidum* has a low infectious dose (57 organisms) by injection. Parenteral inoculation and contact of mucous membranes or broken skin with infectious clinical materials are the primary hazards to laboratory personnel.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of blood or other clinical specimens from humans or infected animals. ABSL-2 practices, containment equipment, and facilities are recommended for work with infected animals.

Special Issues

Vaccines Vaccines are currently not available for use in humans.

Transfer of Agent Importation of this agent requires 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.

Vibrio species

Vibrio species are straight or curved motile Gram-negative rods. Growth of *Vibrio* spp. is stimulated by sodium, and the natural habitats of these organisms are primarily aquatic environments. Though rare in the U.S., cholera is an acute intestinal infection caused by *V. cholerae* with 3–5 million cases and 100,000 deaths each year, globally.[247] There are at least 12 different *Vibrio* spp. isolated from clinical specimens. *V. cholerae* and *V. parahaemolyticus* are common causes of human enteritis, and *V. alginolyticus* and *V. vulnificus* are common causes of extraintestinal infections including wound infections and septicemia.[248] *Vibrio* spp. can easily be isolated using selective and differential media, or can be detected by molecular testing of primary clinical specimens.

Occupational Infections

Rare cases of bacterial enteritis due to Laboratory-associated infections with either *V. cholerae* or *V. parahaemolyticus* have been reported.[84,249–251] Naturally- and experimentally-infected animals and shellfish are potential sources for such illnesses. No other *Vibrio* spp. have been implicated in Laboratory-associated infections.

Natural Modes of Infection

The most common natural mode of infection is the ingestion of contaminated food or water. The human oral infecting dose of *V. cholerae* in healthy, non-achlorhydric individuals is approximately 106–1011 colony-forming units, while that of *V. parahaemolyticus* ranges from 105–107 cells.[252,253] The importance of aerosol exposure is unknown; although, it has been implicated in at least one instance.[251] The risk of infection following oral exposure is increased in persons with abnormal gastrointestinal physiology, including individuals on antacids, with achlorhydria, or with partial or complete gastrectomies. Fatal cases of septicemia may occur in individuals who are immunocompromised or have pre-existing medical conditions such as liver disease, cancer, or diabetes.

Laboratory Safety and Containment Recommendations

Pathogenic *Vibrio* spp. can be present in human fecal samples or in the meats and the exterior surfaces of marine invertebrates such as shellfish. Survival and growth of *Vibrio* spp. in water is dependent on high salinity. Other clinical specimens from which *Vibrio* spp. may be isolated include blood, arm or leg wounds, eye, ear, and gallbladder.[250] LAIs of *V. cholerae* or *V. parahaemolyticus* have been observed in laboratory researchers after the use of syringes, decontamination of a laboratory spill, or the handling of infected animals.[249–251] Exposure of open wounds to *Vibrio* spp. in contaminated seawater or shellfish can result in infections and septicemia.

BSL-2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials. ABSL-2 practices, containment equipment, and facilities are recommended for activities with naturally or experimentally infected animals.

Special Issues

Vaccines A cholera vaccine is licensed and available in the United States. It is currently only recommended for adult travelers to areas of active cholera transmission.[254] There are currently no human vaccines against *V. parahaemolyticus*.

Transfer of Agent Importation of this agent requires 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.

Yersinia pestis

Yersinia pestis, the causative agent of plague, is a Gram-negative bacillus frequently characterized by a “safety pin” appearance on stained preparations from specimens. The incubation period for bubonic plague ranges from two to six days while the incubation period for pneumonic plague is one to six days.

Occupational Infections

Y. pestis is a documented laboratory hazard. A number of LAIs have been reported in the United States, some of which were fatal.[84,255] One lethal case in a laboratory researcher was due to the attenuated strain KIM D27.[256] The condition of hereditary hemochromatosis coupled with diabetes in the researcher is believed to have contributed to the fatal course of disease. Veterinary staff and pet owners have become infected when handling domestic cats with oropharyngeal or pneumonic plague.

Natural Modes of Infection

There is a natural zoonotic cycle of *Y. pestis* between wild rodents and their fleas. Infective fleabites are the most common mode of transmission, but direct human contact with infected tissues or body fluids of animals and humans may also serve as sources of infection.

Plague has a high mortality rate if untreated (50%) and caused three major pandemics, including the Black Death of the 14th century. There are three manifestations of disease: bubonic, septicemic, and pneumonic. Bubonic plague results in tender and painful lymph nodes (buboes). Septicemic plague, which may develop directly or from untreated bubonic plague, can lead to shock and bleeding into the skin and tissues, potentially causing necrosis. Pneumonic plague results in a rapidly developing pneumonia and can be spread from person to person via respiratory droplets. Plague occurs in multiple countries of the world, with the highest incidence in Africa. Most cases in the United States occur in rural, western states. Sporadic cases in the United States average about seven cases per year. Contact with infected sylvatic rodents, such as prairie dogs and ground squirrels, has resulted in human infections.[257]

Laboratory Safety and Containment Recommendations

Y. pestis has been isolated from bubo aspirates, blood, sputum, CSF and autopsy tissues (spleen, liver, lung), depending on the clinical form and stage of the disease; feces, urine or bone marrow samples may be positive for *Y. pestis* DNA or antigen but not the organism itself. Primary hazards to laboratory personnel include direct contact with cultures and infectious materials from humans or animal hosts and inhalation of infectious aerosols or droplets generated during their manipulation. Laboratory animal studies have shown the lethal and infectious doses of *Y. pestis* to be quite low, less than 100 colony-forming units.[258] *Y. pestis* can survive for months in human blood and tissues. Fleas may remain infective for months. It is recommended that laboratory and field personnel be counseled on methods to avoid flea bites and autoinoculation when handling potentially infected live or dead animals.

BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of suspect cultures, animal necropsies, and for experimental animal studies. BSL-3 practices, containment equipment, and facilities are appropriate for production operations. Characterized strains of reduced virulence such as *Y. pestis* strain A1122 can be manipulated at BSL-2. BSL-2 practices, containment equipment, and facilities are recommended for primary inoculation of cultures from potentially infectious clinical materials.

When performing fieldwork involving animals that may have fleas, gloves and appropriate clothing should be worn to prevent contact with skin, and insect

repellent can be used to reduce the risk of flea bites. Arthropod Containment Level 3 (ACL-3) facilities and practices are recommended for all laboratory work involving infected arthropods.[255] See Appendix G for additional information on Arthropod Containment Guidelines.

Special Issues

Be advised of possible misidentification using automated systems. For identification of samples suspected of containing *Y. pestis* using MALDI-TOF MS, it is recommended to use alternative tube extraction that kills viable organisms and not direct spotting of plates in the open laboratory.

Vaccines There are no licensed vaccines currently available in the United States.[259] New plague vaccines are in development but are not expected to be commercially available in the immediate future.[206]

Select Agent *Y. pestis* 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 requires 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-B: Fungal Agents

Blastomyces dermatitidis and *Blastomyces gilchristii*

Blastomyces dermatitidis is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia) that are the infectious particles; conidia convert to large budding yeasts under the appropriate culture conditions *in vitro* at 37°C and in the parasitic phase *in vivo* in warm-blooded animals. Infections with *B. dermatitidis* occur when conidia are inhaled or when yeast forms are injected. The sexual stage is an Ascomycete with infectious ascospores. *Blastomyces gilchristii* was recently recognized as a novel species found predominantly in northwestern Ontario, Wisconsin, and Minnesota.[1]

Occupational Infections

Three groups are at greatest risk of Laboratory-associated infection (LAI): microbiologists, veterinarians, and pathologists.[2] Laboratory-associated local infections have been reported following accidental parenteral inoculation with infected tissues or cultures containing yeast forms of *B. dermatitidis*.[3–9] Laboratory infections have also occurred following the presumed inhalation of conidia from mold-form cultures.[10,11] Infection with *B. dermatitidis* can be pulmonary, cutaneous, or disseminated. Disseminated blastomycosis usually begins with pulmonary infection. Transmission occurs rarely via animal bites, sexual means, or vertical transmission. Forestry workers and other workers with outdoor occupations have developed blastomycosis after exposure to contaminated soil or plant material, particularly moist soil with decaying vegetation.[12] At least 11 reported LAIs with two fatalities have occurred.[13,14]

Natural Modes of Infection

The fungus has been reported in multiple geographically separated countries, but it is best known as a fungus endemic to North America and in association with plant material in the environment. Infections are not communicable but require common exposure from a point source. Although presumed to dwell within the soil of endemic areas, *B. dermatitidis* is extremely difficult to isolate from soil. Outbreaks associated with the exposure of people to decaying wood have been reported. However, outdoor activities were not a risk factor in the largest outbreak reported through 2017; instead, the large Hmong population in the area of Wisconsin that was involved in the outbreak may have had an underlying genetic predisposition.[15] *B. dermatitidis* infections are most common in humans and dogs though other animals, such as cats and horses, may also develop blastomycosis. Human-to-human transmission occurs rarely via perinatal or sexual transmission.

Laboratory Safety and Containment Recommendations

Yeast forms may be present in the tissues of infected animals and in clinical specimens. Parenteral (subcutaneous) inoculation of these materials may cause local skin infection and granulomas. Mold-form cultures of *B. dermatitidis* containing infectious conidia and processing of soil or other environmental samples may pose a hazard of aerosol exposure.

BSL-3 practices, containment equipment, and facilities are recommended for handling sporulating mold-form cultures already identified as *B. dermatitidis* and soil or other environmental samples known or likely to contain infectious conidia.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials, animal tissues, yeast-form cultures, and infected animals.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Coccidioides immitis and *Coccidioides posadasii*

Coccidioides spp. are endemic to the Sonoran Desert of the western hemisphere including northern Mexico, southern Arizona, central and southern California, and western Texas. In recent decades, *C. immitis* has been divided into two species: *C. immitis* and *C. posadasii*.[16] These species are dimorphic fungal pathogens existing in nature and in laboratory cultures at room temperature as filamentous molds with asexual spores (single-cell arthroconidia three to five microns in size) that are the infectious particles. The arthroconidia convert to spherules under the appropriate culture conditions *in vitro* at 37°C and *in vivo* in warm-blooded animals.

Occupational Infections

Laboratory-associated coccidioidomycosis is a documented hazard of working with sporulating cultures of *Coccidioides* spp.[17–19] Occupational exposure in archeologists and prison employees in endemic regions has been associated with high dust exposure.[20,21] Attack rates for laboratory and occupational exposures where a larger number of spores are inhaled are higher than for non-occupational environmental exposures. Smith reported that 28 of 31 (90%) Laboratory-associated infections in his institution resulted in clinical disease, but more than half of infections acquired in nature were asymptomatic.[22] Risk of respiratory infection from exposure to infected tissue or aerosols of infected secretions is very low. Accidental percutaneous inoculation has typically resulted in localized granuloma formation.[23]

Natural Modes of Infection

Single spores in environmental exposures can produce infections by the respiratory route. Peak exposures occur during arid seasons, and exposure can also occur during natural disasters such as earthquakes.[24] *Coccidioides* spp. grow in infected tissue as larger multicellular spherules up to 70 microns in diameter and pose little or no risk of infection from direct exposure.

Most infections from environmental exposure are subclinical and result in life-long protection from subsequent exposures. The incubation period is one to three weeks, and the disease manifests as community-acquired pneumonia with immunologically mediated fatigue, skin rashes, and joint pain. One of the synonyms for coccidioidomycosis is desert rheumatism. A small proportion of infections are complicated by hematogenous dissemination from the lungs to other organs, most frequently skin, the skeleton, and the meninges. Disseminated infection is much more likely in persons with cellular immunodeficiencies (e.g., AIDS, organ transplant recipient, lymphoma, receipt of tumor necrosis factor [TNF] inhibitors) and in pregnant women in the third trimester.

Laboratory Safety and Containment Recommendations

Because of their size, arthroconidia are conducive to ready dispersal in air and retention in the deep pulmonary spaces. The much larger size of the spherule considerably reduces the effectiveness of this form of the fungus as an airborne pathogen.

Spherules of the fungus may be present in clinical specimens and animal tissues, and infectious arthroconidia may be present in mold cultures and soil or other samples from natural sites. Inhalation of arthroconidia from either environmental samples or mold isolates is a serious laboratory hazard.[19] Most exposures occur due to personnel handling cultures of unknown infectious status on the bench, rather than in a BSC. Personnel should be aware that infected animal or human clinical specimens or tissues stored or shipped under temperature and nutrient conditions that could promote germination of arthroconidia pose a theoretical laboratory hazard. Slide cultures should never be prepared from unknown hyaline (colorless) isolates, as they could contain *Coccidioides* spp.

BSL-3 practices, containment equipment, and facilities are recommended for propagating and manipulating sporulating cultures already identified as *Coccidioides* spp. and for processing soil or other environmental materials known or suspected to contain infectious arthroconidia. Experimental animal studies should be done at BSL-3 when challenge is via the intranasal or pulmonary route.

BSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, and processing animal tissues that may contain *Coccidioides* spp. ABSL-2 practices, containment

equipment, and facilities are appropriate for experimental animal studies when the route of challenge is parenteral.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Histoplasma capsulatum

Histoplasma capsulatum is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (macro-and/or microconidia); microconidia are the infectious particles that convert to small budding yeasts under the appropriate culture conditions in vitro at 37°C and in the parasitic phase in vivo. The sexual stage is an Ascomycete with infectious ascospores.

Specific hazards/risks associated with *Histoplasma* include:

1. Immunocompromised individuals are at increased risk of infection and experience more severe infections and higher mortality;
2. Dissemination throughout body has resulted in death but usually results in chronic infection;
3. Previously controlled infections can be re-activated when cellular immunity is impaired;
4. The adrenal gland can be destroyed by visceral infection; and
5. 5–20% of cases involve the central nervous system and appear as chronic meningitis or focal brain lesions.

Occupational Infections

Laboratory-associated histoplasmosis is a documented hazard in facilities conducting diagnostic or investigative work.[9,25–27] Pulmonary infections have resulted from handling mold form cultures.[28,29] Local infection has resulted from skin puncture during autopsy of an infected human,[30] from accidental needle inoculation of a viable culture,[31] from accidental inoculation with a lymph node biopsy sample from an infected patient,[32] and from spray into the eye.[33] Collecting and processing soil samples from endemic areas has caused pulmonary infections in laboratory workers,[34] and one death was reported in 1962.[35] Conidia are resistant to drying and may remain viable for long periods of time. The small size of the infective conidia (less than five microns) is conducive to airborne dispersal and intrapulmonary retention. Work with experimental animals suggests that hyphal fragments are also capable of serving as viable inocula.[25]

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with bird and bat feces. It has been isolated from soil, often in river valleys, between latitudes 45°N and 45°S. Histoplasmosis is naturally acquired by the inhalation of infectious microconidia, which can survive in excess of ten years in soil.[25] Infections are not transmissible from person-to-person but require common exposure to a point source. Large outbreaks have been reported from exposure to soil or plant material contaminated with bird or bat feces[36,37] and from exposure to soil during construction projects.[38]

Laboratory Safety and Containment Recommendations

The infective stage of this dimorphic fungus (microconidia) is present in sporulating mold form cultures and in soil from endemic areas. The yeast form is present in tissues or fluids from infected animals and may produce local infection following parenteral inoculation or splash onto mucous membranes.

BSL-3 practices, containment equipment, and facilities are recommended for propagating sporulating cultures of *H. capsulatum* in the mold form, as well as for processing soil or other environmental materials known or likely to contain infectious conidia.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens; identifying isolates, animal tissues, and mold cultures; identifying cultures that may contain *Histoplasma* in routine diagnostic laboratories; and for inoculating experimental animals, regardless of route. Any culture identifying dimorphic fungi should be handled in a Class II BSC. Protective eyewear should be worn when splash(es) to mucous membranes may occur.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Sporothrix schenckii species complex

The *Sporothrix schenckii* species complex is composed of at least six species (*Sporothrix brasiliensis*, *Sporothrix mexicana*, *Sporothrix globosa*, *S. schenckii sensu stricto, Sporothrix luriei*, and *Sporothrix albicans*) of dimorphic fungal pathogens existing in nature and in laboratory cultures at room temperature as filamentous mold with asexual spores (conidia); the conidia are the infectious particles that convert to small budding yeasts in the parasitic phase in vivo.[39] The sexual stage is unknown.

Occupational Infections

Most cases of sporotrichosis are reported sporadically following accidental inoculation with contaminated material. Large outbreaks have been documented in persons occupationally or recreationally exposed to soil or plant material containing the fungus. However, members of the *S. schenckii* species complex have caused a substantial number of local skin or eye infections in laboratory personnel.[40] Most occupational cases have been associated with accidents and have involved splashing culture material into the eye,[41,42] scratching,[43] injecting infected material into the skin,[44] or being bitten by an experimentally infected animal.[45,46] Skin infections without any apparent trauma to the skin have also resulted from handling cultures[47–49] and from the necropsy of animals.[50]

Laboratory Safety and Containment Recommendations

Although localized skin and eye infections have occurred in an occupational setting, no pulmonary infections have been reported as a result of laboratory exposure. It should be noted that serious disseminated infections have been reported in immunocompromised persons.[51]

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for laboratory handling of clinical specimens suspected of containing infectious particles, soil and vegetation suspected to contain *S. schenckii*, and experimental animal activities with *S. schenckii*. Any culture identifying dimorphic fungi should be handled in a Class II BSC. Protective eyewear should be worn when splash(es) to mucous membranes may occur.

Special Issues

Transfer of Agent Importation of this agent requires 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.

Miscellaneous Yeast and mold organisms causing human infection

The majority of mold organisms in Table 1 cause infection in compromised hosts. Risk factors may include neutropenia, previous exposure to antibiotics, treatment for cancer, especially leukemia and lymphoma, organ or stem cell transplant, severe burns, HIV infection with low CD4 cell counts, and placement of central lines or other monitoring devices.

The majority of these organisms are found in the environment and are transmitted through exposure to air, water, or dust. Mold conidia can be inhaled or injected subcutaneously through trauma or other accidental inoculation. Dermatophytes can be transmitted through the person-to-person route, the animal-to-person route, and the environment-to-person route.

Candida yeasts are found as part of the normal human respiratory or gastrointestinal flora and may cause infection after exposure to antibiotics, abdominal surgery, or other causes. Yeast outbreaks in hospitals can occur through exposure to contaminated hospital equipment, foods, or medications. Some yeast species, most notably *Candida auris*,[52] cause concern because they display resistance to multiple antifungal drugs. *Cryptococcus* basidiospores are found in the environment largely associated with bird droppings or certain trees. They cause infection in compromised hosts after inhaling fungal spores.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for propagating and manipulating cultures known to contain these agents. All unknown mold cultures should be handled in a Class II BSC.

Table 1. Miscellaneous Yeast and Mold

Agent	Occupational Infection	Natural Mode of Infection	Biosafety Level
Candida species	Not common	From point source in environment; from gastrointestinal tract into bloodstream	BSL-2
Cryptococcus neoformans and *C. gattii*	Occasional inoculation into skin when working with laboratory animals	Inhalation from point source in environment. No person-to-person transmission reported.	BSL-2 (handle in BSC to prevent laboratory contamination)
Dermatophyte molds: *Trichophyton, Microsporum, Epidermophyton* species	Occasional direct inoculation from handling isolates or contaminated materials	Person-to-person; common exposure to a point source; handling infected animals	BSL-2
Hyaline Molds: *Aspergillus* spp., *Fusarium* spp.	Not common	Presumed inhalation; subcutaneous inoculation from environmental source	BSL-2 (handle in BSC to prevent laboratory contamination)
Talaromyces (Penicillium) marneffei	Occasional direct inoculation when working with laboratory animals; rare inhalation in immunocompromised individual	Mostly inhalation (in immunocompromised hosts)	BSL-2 (handle in BSC to prevent laboratory contamination)

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Agent	Occupational Infection	Natural Mode of Infection	Biosafety Level
Dematiaceous Molds: *Bipolaris* spp.; *Cladophialophora bantiana; Exophiala* spp*; Exserohilum rostratum; Fonsecaea* spp.*; Pseudallescheria* spp.*; Rhinocladiella* spp.*; Scedosporium* spp.*; Verruconis (Ochroconis) gallopava*	Not reported, but inhalation or subcutaneous inoculation are possible routes of exposure	Presumed inhalation; subcutaneous inoculation from environmental source. *C. bantiana, E. dermatitidis, V. gallopava*, and *R. mackenziei* are neurotropic. *C. bantiana* can cause disseminated infection in otherwise healthy hosts.	BSL-2 (handle in BSC to prevent laboratory contamination)
Mucormycete molds: *Mucor* spp.; *Rhizopus* spp.; *Rhizomucor* spp.; *Lichtheimia (Absidia)* spp.	Not reported	Presumed inhalation; subcutaneous inoculation from environmental source; ingestion	BSL-2 (handle in BSC to prevent laboratory contamination)

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Section VIII-C: Parasitic Agents

General Issues

This section focuses on potential hazards of working in settings in which exposures to viable parasites could occur, and approaches to decrease the likelihood of accidental exposures. Available data are limited; the perspective provided is based on review of the literature regarding reported cases of occupationally-acquired parasitic infections, available information for selected parasites regarding potential intervention measures (e.g., disinfection approaches), and knowledge about parasite biology and about the epidemiology and clinical aspects of parasitic infections. Additional details regarding occupationally-acquired cases of parasitic infections and recommendations for post-exposure management are available elsewhere,[1–3] as is further perspective about zoonoses of occupational health importance in laboratory animal research.[4] Information about diagnosing and treating parasitic infections and perspective regarding special considerations for persons who are immunocompromised or pregnant can be obtained from various reference materials, including the website of CDC's Division of Parasitic Diseases and Malaria, and are available at https://www.cdc.gov/parasites. Diagnostic resources and information about parasitic life cycles, including routes of transmission, are available through CDC's DPDx website at https://www.cdc.gov/dpdx.

Note: Microsporidia historically were considered parasites but are now recognized by most experts as fungi. However, because of their traditional association with parasitology, microsporidia are discussed in Section VIII-C: Parasitic Agents.

Blood and Tissue Protozoal Parasites

In descending order of total number of reported cases of infection reported in the literature, the blood and tissue protozoal parasites that have been associated with documented cases of occupationally acquired infection are: *Trypanosoma cruzi*, *Plasmodium* spp., *Toxoplasma gondii*, *Leishmania* spp., and *Trypanosoma brucei* subspp.[1] Other blood/tissue protozoa of potential concern include *Babesia* spp., the free-living amebae, including *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia pedata*; and the *Sarcocystis* spp. that can cause intramuscular sarcocystosis. In addition, various genera/species of microsporidia (now classified as fungi) may pose an occupational risk for extraintestinal infection; see below regarding an occupationally-acquired case of microsporidiosis.

In alphabetical order: *Leishmania* spp. cause various syndromes, including visceral, cutaneous, and mucosal leishmaniasis (clinical presentation is in part species dependent); *Plasmodium* spp. cause malaria; *T. gondii* causes toxoplasmosis; *T. cruzi* causes American trypanosomiasis (Chagas disease); and *T. brucei* subsp. *gambiense* and subsp. *rhodesiense* cause human African trypanosomiasis

(sleeping sickness). Depending in part on parasite and host factors, infective stages of these parasites may be found in the bloodstream, either briefly (e.g., during a particular phase of the infection), intermittently, or during all or most of the course of the infection. Among these parasites, tissue tropisms vary by genus and species, including which, if any, tissues/organs may become infected and whether the tissue and blood stages of the parasite differ. Some of these pathogens have been reported to be transmitted via blood transfusion, organ/tissue transplantation, and congenitally.[5–7]

Occupational Infections

Occupationally-acquired cases of infection with *Leishmania* spp., *Plasmodium* spp., *T. gondii*, and *Trypanosoma* spp. have been reported. The most commonly reported modes of transmission have included sharps (e.g., needlestick) injuries and other percutaneous exposures (e.g., through preexisting cuts, breaks, or microabrasions).[1,2] Vector-borne transmission to laboratorians has been reported, particularly for *Plasmodium* spp. (*P. falciparum*, *P. vivax*, and the simian parasite *P. cynomolgi*) but also for *T. cruzi* and *Leishmania* major.[1] Other reported laboratory routes of transmission have included mucous membrane exposures (*T. gondii*, *Leishmania* spp., and *T. cruzi*) and ingestion (*T. gondii*).[1,2] Laboratory-associated cases of infection with *Leishmania* spp., *T. gondii*, and *T. cruzi* have also been reported in persons who were working with these organisms but did not recall a discrete accident or exposure.[1,2]

Laboratory-associated cases of infection with blood/tissue protozoa may range from asymptomatic to severe. One individual with a reported case of laboratory-associated *Leishmania* infection developed clinical manifestations consistent with visceral involvement (e.g., fever, splenomegaly, leukopenia);[1,2] this case was caused either by *L. donovani* or by *L. infantum*, which is in the *L. donovani* species complex. The other laboratorians with reported cases of occupationally-acquired *Leishmania* infection (including, but not limited to, the other persons infected with parasites in the *L. donovani* species complex) developed skin lesions (cutaneous leishmaniasis), with or without associated lymphadenopathy.[1,2] One of the individuals who developed cutaneous leishmaniasis ultimately developed mucosal leishmaniasis as a sequela. In this instance, the etiologic agent was *L. amazonensis*, a species found in parts of South America. Overall, the exposure routes for the reported laboratory-associated cases of *Leishmania* infection have included accidental needlestick injuries, preexisting non-intact skin, mucosal contact, and the bite of an infected sand fly in an insectary.[1]

Occupationally-acquired *Plasmodium* infection may be associated with clinical manifestations such as fever, chills, fatigue, and hemolytic anemia. Malaria may be severe and life-threatening, particularly if caused by *P. falciparum*. Mosquito-transmitted (sporozoite-induced) *Plasmodium* infections have been

documented repeatedly in laboratory settings.[1] The other reported cases of occupationally-acquired *Plasmodium* infection have occurred in persons (including healthcare workers) who had accidental sharps injuries or exposures of non-intact skin.[1,2]

Laboratory-associated *T. gondii* infection may range from asymptomatic to relatively mild (e.g., flu-like symptoms, rash, lymphadenopathy) to life-threatening (e.g., myocarditis and encephalitis). Laboratorians have become infected with *T. gondii* via ingestion of sporulated oocysts from feline fecal specimens, as well as via percutaneous (e.g., through needlestick injuries or non-intact skin) or mucosal contact with tachyzoites or bradyzoites from human or animal specimens (e.g., peritoneal fluid from experimentally infected rodents) or cultures.[1,2]

The clinical manifestations of the acute phase of *T. cruzi* infection may include swelling and redness at the site of exposure, fever, rash, and lymphadenopathy. Life-threatening myocarditis and meningoencephalitis may develop. Approximately 20% to 30% of chronically infected persons ultimately develop clinical manifestations, typically cardiac and less often gastrointestinal (megaesophagus or megacolon). Laboratorians have become infected with *T. cruzi* via percutaneous or mucosal exposures, such as to blood from experimentally infected animals or to feces from infected triatomine bugs.

Infection with *T. b. rhodesiense* (East African) and *T. b. gambiense* (West African), which are vector-borne in nature (see below), may cause swelling and redness at the site of exposure, as well as various clinical manifestations during the hemolymphatic stage of the infection. East African trypanosomiasis typically is associated with a more acute course than the West African form, with early invasion of the central nervous system (CNS). After the parasite (of either subspecies) invades the CNS, the infection typically is fatal unless treated. Laboratorians have become infected with *T. brucei* subspp. through sharps injuries or non-intact skin.[1,2]

Various genera/species of microsporidia found naturally in non-human animals can cause extraintestinal infection in humans. Tissue tropisms vary by genus/species and also may be affected by host factors. Spores (i.e., the infective form) of microsporidia are hardy and can survive for long periods in the environment; ingestion is the primary route of transmission in nature, whereas other exposure routes could cause infection in laboratory settings. The one reported laboratory-associated case of microsporidiosis—a case of keratoconjunctivitis without systemic symptoms—occurred in an immunocompetent laboratorian who was accidentally exposed to *Encephalitozoon cuniculi* “when several drops of culture supernatant containing several million spores were spilled into both eyes.”[8]

No laboratory-associated cases of intramuscular sarcocystosis have been reported. However, humans who ingest fecally shed oocysts or sporocysts of

Sarcocystis nesbitti or of various unidentified *Sarcocystis* spp. with unknown carnivorous definitive hosts may develop intramuscular cysts.[9]

Babesia microti and other *Babesia* spp., which can cause human babesiosis (piroplasmosis), are transmitted in nature by the bite of an infected tick. Although no laboratory-associated cases of *Babesia* infection have been reported, such cases could be acquired through percutaneous contact with contaminated blood from infected persons or animals or, for culturable *Babesia* spp., with cultured parasites. Bites from naturally or experimentally infected ticks may also pose a risk.

Among the free-living amebae (FLA), *Naegleria fowleri* causes primary amebic meningitis, which typically progresses rapidly and causes death, whereas *Acanthamoeba* spp., *B. mandrillaris*, and *S. pedata* may cause granulomatous amebic encephalitis, which typically is more subacute or chronic. FLA may also cause disfiguring skin lesions (*Acanthamoeba* spp. and *B. mandrillaris*) and potentially blinding keratoconjunctivitis, particularly in association with the use of contact lenses or the presence of corneal abrasions (*Acanthamoeba* spp.). No laboratory-associated cases of infection with FLA have been reported. However, potentially infective stages of FLA may be found in tissue, cerebrospinal fluid, and other types of specimens from infected persons and in laboratory cultures of the organisms.

Natural Modes of Infection

Leishmania spp., *Plasmodium* spp., and American and African trypanosomes are transmitted in nature by blood-sucking insects. Sandflies in the genera *Phlebotomus* and *Lutzomyia* transmit *Leishmania* spp.; mosquitoes in the genus Anopheles transmit *Plasmodium* spp.; triatomine bugs, including *Triatoma*, *Rhodnius*, and *Panstrongylus* spp., transmit *T. cruzi*, which is found in the feces rather than the saliva of the bugs; tsetse flies in the genus *Glossina* transmit African trypanosomes; and ixodid (hard) ticks transmit *Babesia* spp.

Malaria is widely distributed in the tropics, although the prevalence and incidence rates of *Plasmodium* infection vary in and among areas of endemicity. In aggregate, seven *Plasmodium* spp. have been documented to infect humans in nature, primarily *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* but also the simian species *P. knowlesi*, *P. cynomolgi*, and *P. simium*.

Leishmaniasis is endemic in parts of the tropics, subtropics, and southern Europe. Many *Leishmania* spp. are zoonotic (e.g., have rodent or canine reservoir hosts); however, infected humans serve as epidemiologically important reservoir hosts in some settings for some species, including *L. donovani* and *L. tropica*. Only cats and other felines can serve as definitive hosts for *T. gondii*, which is found worldwide. Birds and mammals, including sheep, pigs, rodents, cattle, deer, and humans, can become infected via ingestion of tissue cysts or mature

(sporulated) fecal oocysts and subsequently develop tissue cysts (e.g., in skeletal muscle, myocardium, brain, eyes). Chagas disease is endemic in Mexico, Central America, and South America; sporadic vector-borne cases also occur in focal areas of the southern United States. Various domestic and wild mammals are found naturally infected with *T. cruzi*. African trypanosomiasis is endemic in sub-Saharan Africa but is highly focal in its distribution. *T. b. gambiense* occurs in parts of western and central Africa, whereas *T. b. rhodesiense* occurs in parts of eastern and southern Africa. *T. b. rhodesiense* is a zoonotic infection with cattle or, in a more limited role, game animals serving as reservoir hosts, whereas humans are the only epidemiologically important hosts for *T. b. gambiense*. *Babesia* infections are found worldwide in animals, and multiple *Babesia* spp. have been documented to infect humans; examples of animal reservoir hosts include white-footed mice (*Peromyscus leucopus*) and other small mammals for *B. microti* and cattle for *B. divergens*.

Laboratory Safety and Containment Recommendations

BSL-2 and ABSL-2 practices, including containment equipment/facilities and laboratory personal protective equipment (PPE), are recommended for activities involving infective stages of the parasites discussed in this section.

Depending in part on the parasite and the phase of the infection, infective stages of blood and tissue protozoa may be present in blood and various body fluids and tissue specimens, including in cultures and homogenates, from infected humans and from experimentally or naturally infected animals, including arthropod vectors if pertinent. See above regarding the primary laboratory hazards. The risks for accidental exposures and occupationally-acquired infections in persons working with cultures, tissue homogenates, blood, or other specimens that contain any of the organisms discussed here, including during procedures that might create aerosols or droplets, should be reduced by use of PPE (e.g., long-sleeved laboratory coat/gown, gloves, face shield, sturdy closed footwear, clothing that covers exposed legs), in conjunction with containment in a biosafety cabinet (BSC). For work with infected arthropod vectors, the prevention measures include using the relevant PPE, as well as maintaining and transporting vectors in facilities or transport containers that reasonably preclude the exposure of personnel or the escape of the arthropods. See Appendix E for additional information.

Special Issues

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

Intestinal Protozoal Parasites

Intestinal protozoal parasites that pose an occupational risk include *Cryptosporidium* spp., which cause cryptosporidiosis; *Cyclospora cayetanensis*, which causes cyclosporiasis; *Cystoisospora belli*, which causes cystoisosporiasis; *Entamoeba histolytica*, which causes intestinal and extraintestinal (e.g., liver abscess) amebiasis; *Giardia duodenalis*, which causes giardiasis; and *Sarcocystis* hominis (from beef) and *S. suihominis* (from pork), which cause intestinal sarcocystosis[9] (see above regarding *Sarcocystis* spp. that can cause intramuscular sarcocystosis). *Dientamoeba fragilis* (for which a cyst stage recently was identified)[10] and *Blastocystis* spp.[11] are additional intestinal protozoal parasites that may pose risk to laboratory workers, although their pathogenic potentials in humans continue to be debated.[10,12] Multiple genera/species of microsporidia (now classified as fungi) can cause intestinal microsporidiosis in humans.

Occupational Infections

Laboratory-associated infections with *Cryptosporidium* spp., *E. histolytica*, *G. duodenalis*, and *C. belli* have been reported.[1–3] The reported cases typically have been associated with ingestion of the parasite and, if symptomatic, with gastrointestinal symptoms. Laboratory work that does or may entail exposure to *Cryptosporidium* oocysts warrants special care. Occupationally-acquired infections have occurred quite commonly in personnel working with this agent, especially if infected calves were the source of the oocysts.[1,2] Other infected animals pose potential risks as well. Circumstantial evidence suggests that airborne transmission of oocysts via droplets of this small organism (i.e., 4–6 µm in diameter) might occur.[1,2] Rigid adherence to protocol (see below) should reduce the risks for accidental exposures and occupationally-acquired infections in laboratory and animal care personnel.

Natural Modes of Infection

All of these intestinal protozoa have cosmopolitan distributions. In nature, the primary route of transmission is ingestion of an environmentally hardy oocyst (for the coccidia), cyst (for *E. histolytica* and *G. duodenalis*), or spore for the microsporidia. The ID50 has been best established for the zoonotic species *Cryptosporidium parvum*: the reported ID50 has ranged from 12 to 2,066 ingested oocysts, depending on the strain tested;[13] and the ID50 for one strain of *C. hominis* ranged from 10 to 83 oocysts.[14] Because intestinal protozoa multiply in the host, ingestion of even small inocula could cause infection and illness. The role, if any, for non-human reservoir hosts differs among the intestinal protozoa. Cattle, other mammals, and birds can be infected with various *Cryptosporidium* spp.

Humans are the primary hosts for *E. histolytica* and *C. belli* and are the only established hosts for *C. cayetanensis*. Most human cases of *G. duodenalis* infection likely are acquired via direct or indirect human-to-human transmission,

although zoonotic transmission may rarely occur, particularly from companion cats and dogs. The parasites discussed in this paragraph do not require more than one host to complete their life cycle.

Laboratory Safety and Containment Recommendations

BSL-2 and ABSL-2 practices, including containment equipment/facilities and laboratory personal protective equipment (PPE), are recommended for activities involving infective stages of the parasites discussed in this section.

Depending on the organism, infective stages of these parasites and of microsporidia may be present in the feces and/or in other body fluids (e.g., bile) and tissues. Appropriate standard precautions are recommended, with special attention to personal hygiene (e.g., handwashing), the use of PPE, and laboratory practices that reduce the risk for accidental ingestion of these organisms. Use of a BSC and/or face shield should also reduce the possibility of airborne transmission via contaminated droplets (e.g., when working with liquid suspensions of *Cryptosporidium* oocysts). *Cryptosporidium* oocysts are infectious when shed in stool because they have already fully sporulated and do not require further development outside the host; the oocysts are often present in high numbers in stool and are environmentally hardy. In contrast, the oocysts of *Cystoisospora belli* and *Cyclospora cayetanensis* require an extrinsic maturation period to become infective, which, under favorable environmental conditions, may be relatively short (potentially, <24 hours) for *C. belli* but is quite long (typically, at least 1–2 weeks) for *C. cayetanensis*.

For disinfection of contaminated surfaces (e.g., benchtops and equipment), commercially available iodine-containing disinfectants are effective against *E. histolytica* and *G. duodenalis*, when used as directed, as are high concentrations of chlorine (one cup of full-strength commercial bleach [~5% chlorine] per gallon of water [1:16, vol/vol]).[1,2] Because undiluted 3% (10 volumes) commercial hydrogen peroxide is known to kill *Cryptosporidium* oocysts after a sufficiently long contact time (data for *Cystoisospora* and *Cyclospora* oocysts are not available), the following approach can be used to decontaminate a surface affected by a laboratory spill containing *Cryptosporidium* oocysts.[1] After removing organic material from the contaminated surface (e.g., by using a conventional laboratory detergent/cleaner) and absorbing the bulk of the spill with disposable paper towels, flood and completely cover the surface with undiluted hydrogen peroxide. Dispense hydrogen peroxide repeatedly, as needed, to keep affected surfaces covered and wet/moist for approximately 30 minutes. Absorb residual hydrogen peroxide with disposable paper towels, and allow surfaces to dry thoroughly (10 to 30 minutes) before use. Care should be taken to autoclave or similarly disinfect all paper towel litter and other disposable materials before disposal. Reusable laboratory items can be disinfected and washed in a laboratory dishwasher by using the sanitize cycle and a detergent containing

chlorine. Alternatively, contaminated items may be immersed for approximately one hour in a water bath preheated to 50ºC and washed thereafter in a detergent/disinfectant solution.

Special Issues

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

Cestode Parasites

Cestode parasites that pose an occupational risk include *Echinococcus* spp., *Hymenolepis (Rodentolepis) nana*, and *Taenia solium*. Echinococcosis is caused by cestodes in the genus *Echinococcus*: *E. granulosus* causes cystic echinococcosis, *E. multilocularis* causes alveolar echinococcosis, and *E. vogeli* and *E. oligarthrus* cause polycystic echinococcosis. Humans serve as intermediate hosts and harbor the metacestode or larval stage, which produces a hydatid cyst. *Hymenolepis nana*, the dwarf tapeworm, is cosmopolitan in distribution and causes hymenolepiasis, which is intestinal infection with the adult tapeworm. *Taenia solium*, the pork tapeworm, causes *taenia*sis, which is the infection of the intestinal tract with the adult worm, and cysticercosis, which is the development of larval/tissue cysts (i.e., cysticerci) in various parts of the body, such as brain and subcutaneous tissue.

Occupational Infections

No Laboratory-associated infections with any cestode parasite have been reported.

Natural Modes of Infection

H. nana may act as a one-host parasite and does not require maturation in an intermediate host. *H. nana* is directly transmissible by ingestion of eggs shed in the feces of definitive hosts (i.e., infected humans or rodents). The life cycles of *Echinococcus* and *Taenia* spp. require two hosts. Canids, including dogs, wolves, foxes, coyotes, and jackals, serve as definitive hosts for *E. granulosus*; and various herbivores, such as sheep, cattle, deer, and horses, serve as intermediate hosts. Foxes and coyotes are the principal definitive hosts for *E. multilocularis*, although various canids and felids also can become infected. Rodents serve as intermediate hosts. Bush dogs and pacas serve as the definitive and intermediate hosts, respectively, for *E. vogeli*. Dogs also may be infected. Wild felines, including cougars, jaguarondi, jaguars, ocelots, and pampas cats, are the definitive hosts for *E. oligarthrus*. Various rodents, such as agoutis, pacas, spiny rats, and rabbits, serve as intermediate hosts. Humans become infected with

Echinococcus spp. when eggs shed by definitive hosts are accidentally ingested. For *T. solium*, humans serve as definitive hosts (i.e., harbor the adult tapeworm) but also may serve as accidental intermediate hosts (i.e., harbor cysticerci, larval/tissue cysts). Pigs, which are the usual intermediate hosts, become infected as they scavenge human stool that contains *T. solium* eggs.

Laboratory Safety and Containment Recommendations

Infective eggs of *Echinococcus* spp. may be present in the feces of carnivore definitive hosts.[4] *E. granulosus* poses the greatest risk because it is the most common and widely distributed *Echinococcus* sp. and because dogs are the primary definitive hosts. For *T. solium*, infective eggs in the feces of humans serve as the source of infection; accidental ingestion of infective eggs is the primary laboratory hazard. Ingestion of cysticerci of *T. solium* or *Taenia* asiatica in pork and *T. saginata* in beef could cause human intestinal infection with the adult tapeworm. Ingestion of the eggs of *H. nana* shed in the feces of definitive hosts (humans or rodents) could result in intestinal infection.

Although no Laboratory-associated infections with *Echinococcus* spp. or *T. solium* have been reported, the consequences of such infections could be serious. For echinococcal infections, the severity and nature of the signs and symptoms, if any, depend in part on the location of the cysts, their size, and condition (alive vs. dead). Clinical manifestations associated with a liver cyst could include hepatosplenomegaly, abdominal pain, and nausea, whereas a lung cyst may cause chest pain, dyspnea, and hemoptysis. For *T. solium*, ingestion of eggs from human feces can result in cysticercosis. Subcutaneous or intramuscular *T. solium* cysts may be asymptomatic; although cysts in the CNS also may be asymptomatic, they may cause seizures and other neurologic manifestations.

For laboratory work with infective stages of the cestode parasites discussed here, BSL-2 and ABSL-2 practices, including containment equipment/facilities and laboratory personal protective equipment (PPE), are recommended, with special attention to personal hygiene (e.g., handwashing), the use of PPE, and laboratory practices that reduce the risk for accidental ingestion of infective eggs. For example, gloves should be worn when there may be direct contact with feces or with surfaces contaminated with fresh feces either from carnivores potentially infected with *Echinococcus* spp., humans potentially infected with *T. solium*, or humans or rodents potentially infected with *H. nana*.

Special Issues

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

Trematode Parasites

The trematode parasites that pose the greatest occupational risk are the *Schistosoma* spp., although others, including *Fasciola* spp., are of concern. *Schistosoma mansoni* causes intestinal schistosomiasis. The adult flukes typically reside in the venules of the bowel and rectum. *Fasciola hepatica*, the sheep liver fluke, causes fascioliasis, in which the adult flukes live in the bile ducts of the human or animal host.

Occupational Infections

Laboratory-associated infections with *S. mansoni* and *F. hepatica* (one possible such case) have been reported, but accidental infections with other *Schistosoma* spp. could also occur.[1,2] Laboratory-associated infections with *F. hepatica* may be asymptomatic or associated with various clinical manifestations, such as right upper quadrant pain, depending in part on the phase of the infection. Most laboratory exposures to schistosomes would result in low worm and egg burdens, with low-risk for long-term morbidity, although acute infection may be associated with clinical manifestations (e.g., dermatitis, fever, cough, hepatosplenomegaly, lymphadenopathy).

Natural Modes of Infection

F. hepatica has a cosmopolitan distribution and is most common in sheep-raising areas; other natural hosts include goats, cattle, hogs, deer, and rodents. Snails in the family *Lymnaeidae*, primarily species of *Lymnaea*, serve as intermediate hosts for *F. hepatica* and release cercariae that encyst on vegetation. Humans become infected with *F. hepatica* by eating raw or inadequately cooked vegetation, especially green leafy plants, such as watercress, on which metacercariae have encysted. The same route of transmission is applicable to *Fasciola gigantica* (giant liver fluke) and *Fasciolopsis buski* (an intestinal fluke). Infection with other trematodes requires consumption of the infected intermediate host (mainly fish or crustaceans); therefore, the laboratory risk posed by these pathogens is minimal if appropriate standard precautions are followed, including the use of PPE.

S. mansoni is endemic in parts of Africa, South America, and the Caribbean. Free-swimming cercariae in contaminated bodies of water infect humans via skin penetration. The natural snail hosts capable of supporting development of *S. mansoni* are various species of *Biomphalaria*.

Laboratory Safety and Containment Recommendations

Infective stages of *F. hepatica* (metacercariae) and *S. mansoni* (cercariae) may be found, respectively, encysted on aquatic plants or free-living in the water in laboratory aquaria used to maintain snail intermediate hosts. Ingestion of fluke metacercariae and skin penetration by schistosome cercariae are the primary laboratory hazards. Dissection or crushing of schistosome-infected snails may

also result in exposure of skin or mucous membranes to cercariae-containing droplets. Additionally, metacercariae may be inadvertently transferred from hand to mouth by fingers or gloves, following contact with contaminated aquatic vegetation or aquaria.

All of the reported cases of laboratory-associated schistosomiasis have been caused by *S. mansoni*, which probably in part reflects the fact that a laboratory life cycle for *S. mansoni* can be maintained using mice, which is not possible for the other *Schistosoma* spp. However, accidental infection with *S. haematobium*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, or *S. guineensis* could easily occur via transdermal penetration if infected snail intermediate hosts are kept in aquaria or if laboratorians work with water samples that contain infective cercariae. In addition, exposure to cercariae of non-human (e.g., avian) species of schistosomes may cause mild-to-severe dermatitis (i.e., swimmer's itch).

BSL-2 and ABSL-2 practices, including appropriate PPE and containment equipment/facilities, are recommended for laboratory work with infective stages of the trematode parasites discussed here (i.e., when there may be direct contact with water containing cercariae or vegetation with encysted metacercariae from naturally or experimentally infected snail intermediate hosts). For example, in addition to gloves, long-sleeved laboratory coats and face shields or other protective garb should be worn when working in the immediate area of aquaria or other water sources that may contain schistosome cercariae. Cercariae can be killed on contact with 70% ethanol.[15] Therefore, precautionary measures include having squirt bottles that contain 70% ethanol as well as bottles that contain hand sanitizers for which alcohol is the active ingredient strategically placed around the laboratory to facilitate immediate access after accidental spills/exposures.[15] Various approaches (e.g., ethanol, bleach, heat) can be used to kill snails and cercariae in the water of laboratory aquaria before discharge to sanitary sewers. For example, heating the water to ≥50°C will kill the cercariae within seconds.[15]

Special Issues

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

Nematode Parasites

Nematode parasites that pose an occupational risk include the ascarids; *Strongyloides stercoralis*; hookworms (both human and animal); *Enterobius vermicularis* (human pinworm); and the human filariae, primarily *Wuchereria bancrofti* and *Brugia* spp. Three hookworm species cause patent disease in humans: *Necator americanus*, *Ancylostoma duodenale*, and *Ancylostoma ceylanicum* (which also

causes patent disease in cats and dogs). *Ancylostoma braziliense*, *A. caninum*, and *Uncinaria stenocephala* cause hookworm infection in cats and dogs and can also cause cutaneous larva migrans in humans. *Ascaris lumbricoides* causes ascariasis in humans and pigs. *Baylisascaris procyonis* (a parasite of raccoons), *Toxocara canis* (dog reservoir), and *Toxocara cati* (cat reservoir) cause visceral, ocular, and neural larva migrans in humans. Larval anisakid nematodes (in fish and squid) cause anisakiasis. *Trichuris trichiura* (whipworm) causes trichuriasis in humans. *E. vermicularis* (pinworm; humans only) causes enterobiasis (oxyuriasis). *S. stercoralis* (humans and dogs) causes strongyloidiasis; animal *Strongyloides* spp. may cause cutaneous larva migrans. *Angiostrongylus cantonensis* causes eosinophilic meningitis, and *Trichinella* spp. cause trichinellosis.

Occupational Infections

Laboratory-associated infections with human hookworms, *A. lumbricoides*, *E. vermicularis*, and *Strongyloides stercoralis* have been reported.[1–3] Laboratory infections with hookworm and *Strongyloides* spp. presumptively acquired from infected animals have also been reported.[1–3] Allergic reactions to various antigenic components of human and animal ascarids and anisakids from fish (e.g., aerosolized antigens) may pose risk to sensitized persons.

Laboratory-associated infections with these nematodes may be asymptomatic or associated with a range of clinical manifestations, depending in part on the parasite species and the location(s) of the parasite in the host. The clinical manifestations of infection with *A. lumbricoides* may include cough, fever, and pneumonitis as larvae migrate through the lungs; the larvae develop into adult worms in the small intestine. Infection with *E. vermicularis* usually causes perianal pruritus, with intense itching.

Natural Modes of Infection

Human hookworm and *S. stercoralis* infections are acquired via transdermal penetration of the skin by infective filariform larvae. These nematodes are commonly found in tropical and subtropical regions of the world and cause infection in the small intestine. In contrast to hookworms, *S. stercoralis* is autoinfective and infection may be lifelong if untreated. Intradermal migration of *S. stercoralis* larvae can be associated with a rapidly moving, serpiginous, pruritic eruption referred to as larva currens (“racing” or “running” larva). The time required for *Strongyloides* larvae passed in stool to develop into infective filariform larvae may be as short as approximately two days (i.e., 48 hours); the time required for hookworm larvae to become infective may be as short as three days.

Human cutaneous larva migrans (creeping eruption) occurs when infective larvae of animal hookworms (typically dog and cat hookworms) or of animal *Strongyloides* spp. penetrate the skin and begin wandering. Hookworm infections in dogs and cats and *Strongyloides* spp. infections in animals are endemic worldwide.

A. caninum larvae can also cause infection if ingested. On rare occasions, ingested *A. caninum* larvae have developed into non-gravid adult worms in the human gut, leading to eosinophilic enteritis.

A. lumbricoides and *T. trichiura* infections are endemic in tropical and subtropical regions of the world. *T. canis* and *T. cati* are found worldwide in dogs and cats, respectively. *B. procyonis* is found primarily in raccoons but may also infect dogs. All of these parasites are transmitted via ingestion of embryonated (larvated) eggs. Unembryonated eggs passed in the stool require 2–3 weeks to larvate and become infectious. The eggs are very hardy in the environment and are resistant to most disinfectants (see below).

E. vermicularis is found worldwide, but pinworm infection tends to be more common in school-age children than adults and in temperate than tropical regions. Pinworm infection is acquired by ingestion of eggs (e.g., eggs on contaminated fingers after scratching the perianal skin). Eggs passed by female worms are not immediately infective but require only several hours to become fully infectious. Pinworm infection is of relatively short duration (approximately 60 days on average) unless reinfection occurs.

Some anisakid larvae (*Anisakis* spp., *Pseudoterranova decipiens*, and *Contracecum* spp.) are infective to humans via ingestion. The larvae may be coughed up, be vomited, or form eosinophilic granulomas in the gastrointestinal tract. These nematodes also are antigenic and may cause immediate hypersensitivity reactions (e.g., urticaria, anaphylaxis) when infected fish are ingested.

Laboratory Safety and Containment Recommendations

Eggs and larvae of most nematodes are not infective in freshly passed feces; development to the infective stages may require from less than one day to several weeks, depending in part on the genus/species and the environmental conditions. Ingestion of infective eggs or transdermal penetration by infective larvae are the primary hazards to laboratory staff and animal care personnel.

To minimize the risk for transdermal penetration when working with cultures or fecal specimens that may contain infective hookworm or *Strongyloides* spp. larvae, PPE should be used to cover exposed skin. In an investigation in which *S. stercoralis*–positive stool specimens were reexamined after they had been stored at 4°C for 24, 48, and 72 hours, 23% of the 74 specimens examined still had viable larvae after refrigeration for 72 hours.[16] The following iodine concentrations have been shown to kill infective larvae immersed in an aqueous iodine solution for one to five minutes: 50 ppm iodine for *S. stercoralis* larvae, 60 ppm for *N. americanus* (hookworm) larvae, and 70 ppm for *A. caninum* (hookworm) larvae.[17] In vitro exposure to 70% ethanol has been shown to kill infective *S. stercoralis* larvae within 4.3 ± 1 minutes (mean ± standard deviation).[18] In vitro exposure to 70% ethanol has been shown to kill 95.6% of 45 infective

N. americanus larvae within five minutes and to kill all such larvae within 10 minutes.[19] Taking into consideration the data summarized in this paragraph, Lugol's iodine (1% povidine iodine; 10,000 ppm) may be used to kill *N. americanus* and *S. stercoralis* infective larvae on exposed skin and 70% ethanol (which leaves far less residue on surfaces) may be used to disinfect contaminated laboratory surfaces and equipment.

Ascarid (*A. lumbricoides*, *Toxocara* spp., *B. procyonis*) and *E. vermicularis* eggs are sticky; special care is warranted to ensure that contaminated surfaces and equipment are thoroughly cleaned. Precautions are warranted even when working with formalin-fixed stool specimens. Ascarid eggs, which are exceptionally environmentally resistant, may continue to develop to the infective stage in formalin;[20] they also may continue to develop despite exposure to high concentrations of disinfectants for long periods. However, ascarid eggs can be deactivated by the use of heat at or above 60ºC for more than 15 minutes.

Accidental ingestion of larvated (infectious) eggs of *Toxocara* and *B. procyonis* could lead to visceral migration of larvae, including invasion of the eyes and CNS. The larvae of *Trichinella* in fresh or digested animal tissue, or of *A. cantonensis* in fresh or digested mollusk tissue, could cause infection if accidentally ingested. Vector arthropods infected with filarial parasites pose a potential hazard to laboratory personnel. The prevention measures include using the relevant PPE (e.g., gowns, gloves, closed shoes); maintaining and transporting vectors in facilities or transport containers that reasonably preclude the exposure of personnel or the escape of infected arthropods are also essential. See Appendix E for additional information.

The use of primary containment (e.g., BSC) during work that may be associated with aerosolization should reduce the potential for exposure to aerosolized antigens of ascarids and anisakids, which can cause allergic reactions in sensitized persons. Special attention to use of PPE and to personal hygiene (e.g., handwashing) is warranted when working with any of the nematode pathogens discussed here.

Special Issues

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

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Section VIII-D: Rickettsial Agents

Coxiella burnetii

Coxiella burnetii is a bacterial obligate intracellular pathogen that is the etiologic agent of Q (query) fever. It undergoes its developmental cycle within an acidic vacuolar compartment, exhibiting many characteristics of a phagolysosome. The biphasic developmental cycle consists of a small cell variant (SCV) and a large cell variant (LCV). The SCV is the more structurally-stable cell variant, persisting for extended periods of time outside of host cells and exhibiting resistance to extracellular stresses (drying, extreme temperatures, environmental conditions). The LCV is the larger, metabolically-active variant, which facilitates replication of the agent.[1–4] The organism undergoes a virulent (phase I) to avirulent (phase II) transition upon serial laboratory passage in eggs or tissue culture.

The ID of phase I organisms in laboratory animals has been calculated to be as small as a single organism.[5] The estimated human ID for development of 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 present with pneumonia and hepatomegaly. Infections range from subclinical to severe, and primary/acute infections respond readily to antibiotic treatment. Although rare, *C. burnetii* can cause chronic infections such as endocarditis, granulomatous hepatitis, or vascular infections.[7]

Occupational Infections

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

Natural Modes of Infection

Q fever occurs worldwide. A broad range of domestic and wild mammals are natural hosts for Q fever and may serve as potential sources of infection. Parturient animals and their birth products are common sources of infection. The placenta of infected sheep may contain as many as 10^9 organisms per gram of tissue[12] and milk may contain 10^5 organisms per gram. The resistance of the organism to drying and its low infectious dose can lead to dispersal from contaminated sites. The agent may also be present in infected arthropods, and it may be present in the blood, urine, feces, milk, and tissues of infected animals or human hosts.

Laboratory Safety and Containment Recommendations

Recent advances leading to cell-free media supporting the growth of *C. burnetii*[13] have greatly reduced the necessity of using embryonated eggs or cell culture techniques for propagation and accompanying extensive purification procedures. Exposure to infectious aerosols and parenteral inoculation remain the most likely sources of infection to laboratory and animal care personnel.[8,9]

BSL-3 practices and facilities are recommended for activities involving the inoculation, incubation, and harvesting of *C. burnetii*, the necropsy of infected animals, and the manipulation of infected tissues. Because infected rodents may shed the organisms in urine or feces,[8] experimentally infected animals should be maintained under ABSL-3. A specific plaque-purified clonal isolate of an avirulent (phase II, Nine Mile Strain, plaque purified clone 4) strain is exempt from the Select Agent Regulations and may be safely handled under BSL-2 conditions.[14] BSL-2 practices and facilities are recommended for nonpropagative laboratory procedures, including serological examinations and staining of impression smears.

Special Issues

C. burnetii is among the most environmentally stable of non-spore forming bacteria with a known capacity for extended survival in soil or other contaminated materials, such as animal products, for years.[4] The ID approaches a single organism,[5] thus the capacity for airborne or aerosol transmission is high. Infections are frequently asymptomatic, or cause relatively mild, flu-like symptoms, but can be severe. Chronic infections (i.e., endocarditis) are possible, particularly in those with pre-existing valvular damage or immunocompromised individuals. Q fever is a known hazard during pregnancy.[15]

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 by Spinelli and Bernard.[10,16]

Vaccines Q fever vaccines are not commercially available in the United States. Individuals with valvular heart disease should not work with *C. burnetii*. Work with *C. burnetii* should be avoided during pregnancy. See Section VII for additional information.

Select Agent *C. burnetii* is considered a Select Agent under the Code of Federal Regulations (42 CFR Part 73). All rules concerning the possession, storage, use, and transfer of Select Agents apply. Appendix F contains additional information on Select Agents, including contact information for registration and obtaining appropriate permits for importing, exporting, or transporting this agent.

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

Rickettsia species and *Orientia tsutsugamushi*

Rickettsia prowazekii, *Rickettsia typhi*, the Spotted Fever Group agents of human disease (*Rickettsia rickettsii*, *Rickettsia conorii*, *Rickettsia akari*, *Rickettsia australis*, *Rickettsia sibirica*, and *Rickettsia japonica*), *Orientia tsutsugamushi*, *Rickettsia philipii* (*Rickettsia* 364D), *Rickettsia parkeri*, and various other *Rickettsia* spp. either known as or suspected to be human pathogens of varying pathogenicity are the respective etiologic agents of epidemic typhus, endemic (murine) typhus, Rocky Mountain spotted fever, Mediterranean spotted fever, rickettsialpox, Queensland tick typhus, North Asian spotted fever, Japanese spotted fever, scrub typhus, Pacific Coast tick fever (PCTF), and *Rickettsia parkeri* rickettsiosis.

Rickettsia spp. are bacterial obligate intracellular pathogens that are transmitted by arthropod vectors and replicate within the cytoplasm of eukaryotic host cells. *Rickettsia* spp. are broken into four groups within the genus: the typhus group, the Spotted Fever Group, a transitional group, and an ancestral group.[17] The more distantly related scrub typhus group is now considered a distinct genus, *Orientia*. *Rickettsia*e 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, fleas, or mites.

Occupational Infections

Although not a natural route of infection, some *Rickettsia* spp. can be infectious by an aerosol route, thus adherence to BSL-3 practices is essential. Parenteral inoculation/needlestick injuries are also among the more common routes of laboratory infection. Infections can also be acquired by conjunctival inoculation.

Pike reported 56 cases of epidemic typhus with three deaths, 68 cases of murine typhus, and 57 cases of laboratory-associated typhus (type not specified).[8] Three cases of murine typhus were reported from a research facility.[18] Two of these three cases were associated with the handling of infectious materials on the open bench; the third case resulted from an accidental parenteral inoculation.

Rocky Mountain spotted fever (RMSF) is a documented hazard to laboratory personnel. Pike reported 63 laboratory-associated cases, 11 of which were fatal and occurred prior to 1940.[8] Since that time, two fatalities occurred, in the same facility and presumably from the same exposure, among a laboratory worker and a custodian in 1977. These illnesses were presumed to be employment-related.[19]

Oster reported nine cases occurring from 1971 to 1976 in one laboratory, which were believed to have been acquired as a result of exposure to infectious aerosols.[20]

Natural Modes of Infection

The epidemiology of rickettsial infections is a reflection of the prevalence of the *rickettsia*e in the vector population and the interactions of the arthropod vector with humans. Epidemic typhus is unusual among *rickettsia*e in that humans are considered the primary host. Transmission is by the human body louse, and outbreaks are now associated with breakdowns of social conditions.[21] Under these conditions, even with appropriate treatment, mortality averaged about 4%.[22] Endemic typhus is maintained in rodents and transmitted to humans by fleas. The various spotted fever group *rickettsia*e are limited geographically, probably by the distribution of the arthropod vector (usually ticks), although specific spotted fever group *rickettsia*e are found on all continents.[23]

Laboratory Safety and Containment Requirements

Accidental parenteral inoculation and exposure to infectious aerosols are the most likely sources of Laboratory-associated infection.[24] Aerosol transmission of *R. rickettsii* has been experimentally documented in non-human primates.[25] Five cases of rickettsialpox recorded by Pike were associated with exposure to bites of infected mites.[8]

The tissues of naturally and experimentally infected mammals and their ectoparasites are potential sources of human infection. The organisms are relatively unstable under ambient environmental conditions.

BSL-3 practices and containment equipment are recommended for activities involving culture propagation or specimen preparation and propagation of clinical isolates known to contain or potentially containing *Rickettsia* spp. pathogenic to humans.

Arthropod Containment Level 3 (ACL-3) practices and facilities are recommended for animal studies with arthropods naturally or experimentally infected with rickettsial agents of human disease.[26]

Laboratory work with *Rickettsia* spp. may be conducted in a BSL-2 facility with enhanced special practices including strict access control, competency, and adherence to BSL-3 practices. Laboratories should be locked and access to non-essential personnel should be prohibited. BSL-3 practices include, but are not limited to, appropriate personal protective equipment (e.g., rear-closing gowns, gloves, eye protection, and respiratory protection such as N95 respirators or PAPRs), use of BSCs when handling any open container with potentially infectious material, and primary containment, such as sealed centrifuge rotors

and other means of containment outside the BSC. Disruption of infected cells or yolk sacs should be accomplished within the BSC using an enclosed chamber to minimize the potential for aerosols. If eggs are used for propagation, the site of inoculation should be sealed with an appropriate sealant prior to transfer to an incubator. BSL-2 facilities with BSL-3 practices are recommended for all manipulations of known or potentially infectious materials, including the necropsy of experimentally infected animals and trituration of their tissues, and inoculation, incubation, and harvesting of embryonated eggs or cell cultures. Use of sharps should be minimized. When use of sharps is necessary, they should be disposed of and decontaminated appropriately. All contaminated materials should be effectively decontaminated before removal from the laboratory. If transport to an autoclave is necessary, materials should be double-bagged.

BSL-2 practices and facilities are recommended for nonpropagative laboratory procedures with inactivated samples, including serological and fluorescent antibody procedures, nucleic acid amplification, and for the staining of impression smears after fixation.

ABSL-2 practices and facilities are recommended for the holding of experimentally infected mammals other than arthropods. Several species including *R. montanensis*, *R. rhipicephali*, *R. bellii*, *R. amblyommatis*, and *R. canadensis* are not known to cause human disease and may be handled under BSL-2 conditions. New species are frequently described 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 infection, it is essential that laboratories working with *rickettsia*e have an effective system for reporting febrile illnesses in the laboratory, animal facility, and support personnel; medical evaluation of potential cases; and the institution of appropriate antibiotic therapy when indicated. Prophylactic antibiotic treatment following a potential exposure is discouraged in the absence of clinically compatible signs and symptoms and could delay onset of disease. Vaccines are not currently available for use in humans.

Laboratory Surveillance

Since 1940, only two laboratory fatalities have occurred due to *R. rickettsii*.[19,27,28] This incident emphasizes the necessity of controlling access to the laboratory and expeditious reporting of any exposure or unexplained illness.

Special Issues

Occupational Health Recommendations Under natural circumstances, the severity of disease caused by rickettsial agents varies considerably.[23,29] In the laboratory, very large inocula are possible, which might produce unusual and very serious responses. Surveillance of personnel for Laboratory-associated

infections with rickettsial agents can dramatically reduce the risk of serious consequences of disease. See Section VII for additional information.

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 LAIs is a reliable surveillance system, which includes:

1. Round-the-clock availability of an experienced medical officer knowledgeable about infectious disease;
2. Education of all personnel on signs and symptoms of disease and the advantages of early therapy;
3. A non-punitive, anonymous reporting system for all recognized accidents; and
4. The reporting of all febrile illnesses, especially those associated with headache, malaise, and prostration when no other certain cause exists.

Select Agent *R. prowazekii* is considered a Select Agent under the Code of Federal Regulations (42 CFR Part 73). All rules concerning the possession, storage, use, and transfer of Select Agents apply. Appendix F contains additional information on Select Agents, including contact information for registration and obtaining appropriate permits for importing, exporting or transporting this agent.

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

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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. HFRS caused by Seoul or Seoul-like viruses originating from *Rattus* sp. has been described worldwide. Hantaviruses have been recently described worldwide in shrews, but no human disease has been described yet from these viruses.

Occupational Infections

Documented Laboratory-associated infections have occurred in individuals working with hantaviruses.[1–4] Extreme caution must be used in performing any laboratory operation that may create aerosols (e.g., centrifugation, vortex-mixing). 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. Human infections and illness (caused by Seoul-like virus) have been reported in Europe and the U.S. in people raising and trading pet rats.[7,8] Person-to-person transmission does not occur, with the exception of a few rare instances documented, for Andes virus.[9,10] 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.[4–6,10] 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;[11,12] however, the infectivity of blood or tissues is unknown.

All work involving inoculation of virus-containing material into rodent species permissive for chronic infection should be conducted at ABSL-4. Cell-culture virus propagation and purification should be carried out in a BSL-3 facility using BSL-3 practices, containment equipment, and procedures. Serum or tissue samples from potentially infected rodents should be handled at BSL-2 using BSL-3 practices, containment equipment, and procedures. Potentially infected tissue samples should be handled in BSL-2 facilities following BSL-3 practices and procedures.

BSL-2 practices, containment equipment, and facilities are recommended for laboratory handling of sera from persons potentially infected with hantaviruses. The use of a BSC is recommended for all handling of human body fluids when potential exists for splatter or aerosol. Experimentally infected rodent species known not to excrete the virus can be housed in ABSL-2 facilities using ABSL-2 practices and procedures.

Special Issues

Transfer of Agent Importation of this agent requires 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.

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

Hendra virus (HeV) and Nipah virus (NiV) are members of the genus called *Henipavirus*, within the family *Paramyxoviridae*.[13] Outbreaks of a previously unrecognized paramyxovirus, at first called equine morbillivirus, later named Hendra virus, occurred in horses in Australia in 1994 and 1995. From 1994 to 2017, there have been more than 90 confirmed cases of Hendra virus infection in horses in Queensland and in northeast New South Wales. Following contacts with infected horses, four out of the seven human cases described were fatal and associated with encephalitis or respiratory disease. 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 infected pigs (i.e., pig farmers and abattoir workers).[14–16] A few patients developed a respiratory disease. Approximately 40% of cases resulted in fatalities. Following the 1998–1999 outbreak in Malaysia, the WHO Regional Office for South-East Asia reported 16 outbreaks in Bangladesh and India between 2001 and 2012, totaling 263 cases. Person-to-person transmission of Nipah virus in Bangladesh and India are reported regularly. Transmission also occurs from direct exposure to infected bats and through consumption of raw date palm sap contaminated with infectious bat excretions. In 2014, an outbreak of Nipah virus occurred in the Philippines that resulted in deaths of horses and humans. Outbreaks of Nipah in South-East Asia have a strong seasonal pattern,

occurring between December and May, possibly due to bat breeding season or the date palm sap harvesting season.[17–19] A new henipavirus, Cedar virus, has been isolated from pteropid bats and has significantly reduced virulence in several animal models. The reduced virulence is likely related to alterations found in the P gene, which ablates the production of innate immune antagonist proteins.

Occupational Infections

No Laboratory-associated infections are known to have occurred because of Hendra or Nipah virus exposure. However, people in close contact with Hendra virus-infected horses, especially veterinary professionals (i.e., four cases with two fatalities), are at high risk of contracting the disease.[20–24]

Natural Modes of Infection

The natural reservoir hosts for the Hendra and Nipah viruses appear to be fruit bats of the genus *Pteropus*.[25–27] Studies suggest that a locally occurring member of the genus, *Pteropus giganteus*, is the reservoir for the virus in Bangladesh.[28] Individuals who had regular contact with bats had no evidence of infection (i.e., antibody) in one study in Australia.[29] Human-to-human transmission has been described in familial clusters and associated with close care of severely ill patients.[30]

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, equine blood or body fluids (Australia), or pigs (Malaysia/Singapore), but presumed transmission from *Pteropus* bats to humans via palm date juice has been recorded in Bangladesh. Live virus has been detected in bat urine, implying the important role of urine in transmitting henipaviruses to spillover hosts. 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, and virus was present in secretions of patients, albeit at low levels.[31,32] Active surveillance for infection of healthcare workers in Malaysia has not detected evidence of Laboratory-associated infections in this setting.[33]

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.

Work with Cedar virus in a new animal model should be performed at ABSL-3 until it is demonstrated that the virus does not result in observable illness. Work with Cedar virus in susceptible animal hosts can be performed at ABSL-2 if it has been demonstrated that the virus is avirulent/non-pathogenic and following a risk assessment of the proposed work.

Special Issues

Vaccines Vaccines are not available for use in humans, but Hendra vaccine is available in Australia for horses.

Select Agent Hendra and Nipah virus are Select Agents requiring registration with CDC or USDA for possession, use, storage, and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent requires 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 (HAV) is a positive-sense single-stranded RNA virus, the type species of the Hepatovirus genus in the family *Picornaviridae*. Hepatitis E virus (HEV) is a positive-sense single-stranded RNA virus of the genus Orthohepevirus in the family *Hepeviridae*. There are four major hepatitis E genotypes that infect humans: genotypes 1, 2, 3, and 4.

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 non-human primates.[34] Workers handling other susceptible primates (e.g., owl monkeys, marmosets) also may be at risk for hepatitis A infection. Hepatitis E virus appears to be less of a risk to laboratory personnel than hepatitis A virus, except during pregnancy, when infection with HEV genotype 1 can result in increased maternal and fetal morbidity or mortality. Exposure to HEV-infected pigs, the primary animal reservoir for hepatitis E virus, rabbits, or macaques may pose an occupational hazard to animal handlers, but the extent of this risk is unknown.

Natural Modes of Infection

Most infections with hepatitis A are foodborne and occasionally waterborne. The virus has, on rare occasions, been transmitted through blood, blood-derived products, and other potentially infectious materials. Usually, infectious virus is

present in feces and blood during the incubation period, prodromal phase of the disease, and one week after jaundice onset, but it is not transmitted later in infection and the convalescence period. Hepatitis E virus genotypes 1 and 2 are transmitted via the fecal-oral route primarily by contaminated water in developing countries resulting in sporadic cases and occasionally large outbreaks. Hepatitis E virus genotypes 3 and 4 are associated with zoonotic hepatitis E infections transmitted to humans mainly through consumption of raw or undercooked pork and game meat or by contact with infected animals. This occurs in developed countries and results in sporadic cases. Transmission through blood and blood-derived products has been reported. Infection generally causes an acute self-limiting disease after an incubation period of two to six weeks but chronic infection with genotype 3 has been reported in immunocompromised individuals.

Laboratory Safety and Containment Recommendations

These agents may be present in feces and blood of infected humans and non-human 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 non-human primates. There is no evidence that aerosol exposure results in infection. Although hepatitis A virus is known to be one of the most stable viruses in the environment, hepatitis E virus is also very stable.

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

Special Issues

Vaccines FDA-licensed inactivated vaccines against hepatitis A are available. There are no FDA-licensed vaccines against hepatitis E in the U.S., but a vaccine is currently available in China.

Transfer of Agent Importation of this agent requires 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, Hepatitis D Virus

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

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.[35,36,38]

Hepatitis C virus infection can occur in the laboratory as well.[37] The prevalence of the 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.[39]

Natural Modes of Infection

These viruses are naturally acquired from a carrier during blood transfusion, injection, tattooing, or body piercing with inadequately sterilized instruments. Non-parenteral routes, such as domestic contact and unprotected (heterosexual and homosexual) intercourse, are potential modes of transmission. Vertical transmission (i.e., mother to child) is also possible.

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

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.[40] 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 somewhat stable at room temperature on surfaces or equipment.[41,42] Infectivity of the virus is sensitive to repeated freezing and thawing.

BSL-2 facilities with 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. BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids and tissues. ABSL-2 practices, containment equipment, and facilities are recommended for activities utilizing naturally or experimentally infected chimpanzees or other non-human primates (NHPs). 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.[43]

Special Issues

Vaccines Licensed recombinant vaccines against hepatitis B are available and are highly recommended for laboratory personnel.[35,36,38] 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 requires 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.

Macacine alphaherpesvirus 1 (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, and 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.[44] There have been no reported fatal cases where prompt first aid with wound or exposure site cleansing was performed within minutes after the exposure and post-exposure prophylaxis was given. Reactivated ocular disease has occurred in one individual,[45] and three infections resulting in seroconversion to B virus have occurred in the last decade. Cases prior to 1970 were not treated with antiviral agents because none were available. Morbidity and mortality associated with zoonotic infection result 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 1987–2016, five additional fatal infections brought the number of lethal infections to 21 since the discovery of B virus in 1932.[46]

Occupational Infections

B virus is a hazard in facilities where macaque monkeys are present. Mucosal secretions (i.e., saliva, genital secretions, and conjunctival secretions) are the primary body fluids associated with the risk of B virus transmission. However, it is possible for other materials to become contaminated. For instance, in 1997 a research assistant at the Yerkes Primate Center suffered a mucosal splash without injury while transporting a caged macaque; the individual subsequently died.[47] Based on the work being performed, the activity was considered

low-risk at that time. However, feces, urine, or other fluids and surfaces may be contaminated with virus shed from mucosal fluids. Zoonoses have been reported following virus transmission through a bite, scratch, or splash accident, but in at least two cases, no recognized exposure could be recalled. In one such case, fatality occurred. Multiple 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.[44] However, the risks associated with this hazard are readily reduced by practicing barrier precautions and by rapid and thorough cleansing immediately following possible site contamination. Precautions should be taken when work requires the use of any macaque species, even antibody-negative animals. Animals that are seronegative may be acutely infected and shedding virus but not yet antibody positive. In most documented cases of B virus zoonosis, the 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 three decades underscores that B virus infections have a low probability of occurrence, but when they do occur there are high consequences.

Specific, regular training for B virus hazards, including understanding the modes of exposure and transmission, should be provided to individuals encountering B virus hazards. Training should also include proper use of engineering controls and 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.[47] 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 approximately 10% of newly caught rhesus monkeys have antibodies against the virus, which is frequently present in kidney cell cultures of this animal. In macaque species, the virus can cause vesicular lesions on the tongue and lips and sometimes of the skin. B virus is not present in blood or serum in healthy infected macaques. Transmission of B virus appears to increase when macaques reach sexual maturity.

Laboratory Safety and Containment Recommendations

The National Academies Press published the Institute for Laboratory Animal Research's (ILAR) guidelines for working with non-human primates.[48] The guidelines provide additional information regarding risks and mitigation strategies when handling non-human primates.

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 via mucous membranes or skin breaks provides this agent access to a new host, whether the virus is being shed from a macaque or human, or is present in or on contaminated cells, tissues, or surfaces.[44] 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, the 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-4 facilities are recommended for the propagation of viruses 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 ABSL-4 containment. BSL-3 practices are recommended for handling diagnostic materials with possible B virus. 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.

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.[44]

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 three decades. Guidelines are available for safely working with macaques and should be consulted.[44,49] 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; this is inclusive 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 workplace risk by obscuring vision and contributing to increased risk of bites, needlesticks, scratches, or splashes.

Special Issues

Post-exposure prophylaxis with oral acyclovir or valacyclovir should be considered when exposures are thought to have occurred. Even a slight scratch can result in transmission. Therapy with intravenous acyclovir and/or ganciclovir in documented B virus infections is also important in the reduction of morbidity following B virus zoonotic infection.[44] Ganciclovir is generally reserved for symptomatic cases confirmed by CSF evaluation. 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.[50] There are no effective vaccines available and no curative therapeutics for humans.

Transfer of Agent Importation of this agent requires 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.

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), herpes simplex virus-2 (HSV-2), human cytomegalovirus (HCMV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), and human herpesviruses (HHV) 6A, 6B, 7, and 8.51

Because these viruses establish lifelong latency in human tissues, they may manifest either as primary or recurrent infections. HSV primary and recurrent infections are usually characterized by localized vesicular lesions at or near the site of the initial infection. Primary infection with HSV-1 often occurs in early childhood and may be mild and unapparent. Symptoms such as fever or malaise can sometimes occur. HSV-1 is a frequent cause of viral meningoencephalitis. Genital infections, usually caused by HSV-2, generally occur in adults and are sexually transmissible.

Disseminated disease and encephalitis that may occur in neonatal infections can be fatal. EBV is the most frequent cause of infectious mononucleosis and is also associated with the pathogenesis of several lymphomas and nasopharyngeal cancer.[52,53] EBV-associated cancers normally have viral genomes integrated into the transformed cells. HCMV is often undiagnosed, presenting as a nonspecific

febrile illness with features of infectious mononucleosis. HCMV can cause severe congenital syndrome, which may manifest as mental retardation, microcephaly, motor disabilities, and chronic liver disease in infants who were exposed to the virus in utero.[51] Congenital HCMV is also a frequent cause of deafness in children who were exposed to the virus in utero.

Primary infection with VZV causes chickenpox, while recurrences of this viral infection cause herpes zoster (shingles). Primary infection with HHV-6B or HHV-7 can cause exanthem subitum (roseola), a common childhood rash-associated illness and can also be a cause of infectious mononucleosis syndrome.[53,54] Other clinical manifestations of roseola include nonspecific febrile illness and febrile seizures. Reactivation of HHV-6 is usually identified only in the severely immunocompromised, when it may be associated with encephalitis or other manifestations. Disease caused by HHV-6A, which is a less common infection that usually occurs after early childhood, is less well-understood. HHV-8 is the causative agent of Kaposi's sarcoma and of primary effusion lymphoma.[55] 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.[56] The prevalence of HHV-8 is also higher among intravenous drug users than in the general population.[56] At least one report has provided evidence that, in African children, HHV-8 infection may be transmitted from mother to child.[57]

While few of the human herpesviruses have been demonstrated to cause Laboratory-associated infections, they are both primary and opportunistic pathogens, especially in immunocompromised hosts, in whom recurrent infections can be particularly severe and even life-threatening. Macacine alphaherpesvirus 1 (B-virus, Monkey B virus) is not a human herpesvirus and is discussed separately in the preceding agent summary statement.

Occupational Infections

Few of the human herpesviruses have been documented as sources of Laboratory-associated infections. Although this diverse group of 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. These viruses both infect and establish latency in different types of cells leading to some of the major clinical differences in the disease that they cause. Transmission of human herpesviruses in nature is generally associated with close, intimate

contact with a person excreting the virus in their saliva, urine, or other bodily fluids.[57] For example, VZV is transmitted person-to-person through direct contact, aerosolized vesicular fluids, and respiratory secretions. HHV-8 and CMV can be transmitted through organ transplantation[58,59] and blood transfusion.[60] The ability of HHV-6 to integrate into the human genome allows vertical transmission in a small percentage of cases.

Laboratory Safety and Containment Recommendations

Clinical materials, including blood, urine, and saliva, and isolates of human herpesviruses may pose a risk of infection following ingestion, parenteral inoculation, and droplet exposure of the mucous membranes of the eyes, nose, or mouth, exposure to non-intact skin, or inhalation of concentrated aerosolized materials. Clinical specimens containing the more virulent Macacine alphaherpesvirus 1 (B-virus) may be inadvertently submitted for diagnosis of suspected herpes simplex infection, though the combination of a suspected herpes simplex infection with exposure to a rhesus macaque should trigger serious concern in the treating physician, and ideally would involve special labelling and consultation with the microbiology laboratory. HCMV may pose a special risk to pregnant women because of potential infection of the fetus. All human herpesviruses pose an increased risk to persons who are immunocompromised and are not previously immune to these viruses.

BSL-2 facilities with 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. 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 LAIs, it is prudent to avoid the generation of aerosols during the handling of clinical materials or isolates or during the necropsy of animals.

Autologous transformation of B cells using EBV should not be performed.

Containment recommendations for Macacine alphaherpesvirus 1 (B-virus, Monkey B virus) are described in the preceding agent summary statement.

Special Issues

Vaccines Vaccines for varicella-zoster are 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 reduce the severity of disease.[61]

Treatment Antiviral medications are available for treatment or prevention of infections with several of the human herpesviruses.

Transfer of Agent Importation of this agent requires 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 Viruses

Influenza is an acute viral disease of the respiratory tract. The most common clinical manifestations are fever, headache, malaise, sore throat, cough, and muscle aches. GI tract manifestations (e.g., nausea, vomiting, 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.[62]

Influenza virus infection may be associated with extrapulmonary complications, including viral myocarditis and viral encephalitis. Cardiovascular deaths during influenza epidemics have increased indicating that cardiovascular complications, including exacerbation of chronic underlying conditions, are important contributors to influenza-related morbidity and mortality.[63,64]

Influenza viruses are enveloped RNA viruses belonging to the family *Orthomyxoviridae*. There are four serotypes of influenza viruses—A, B, C, and D, of which human infections have been virologically confirmed for all except influenza D viruses. Influenza A viruses are further classified into subtypes by the surface glycoproteins hemagglutinin (H) and neuraminidase (N). Emergence of new subtypes (antigenic shift) in humans occurs at irregular intervals with Type A viruses. New subtypes can result from reassortment of human, swine, and avian influenza A virus genes. If there is little or no population immunity and the viruses are able to spread in a sustained manner from human-to-human, they can be responsible for rare pandemics. Minor antigenic changes within a circulating seasonal influenza A virus subtype or influenza B virus lineage (antigenic drift) are ongoing processes that are responsible for annual epidemics that make the annual reformulation of influenza vaccines necessary.

Influenza A viruses of different antigenic subtypes occur naturally in many domestic and wild avian species and have formed sustained lineages in swine, equine, and canine species. Avian origin influenza A viruses also sporadically infect multiple other mammalian species. Two influenza A virus subtypes have only been detected in bats. Novel influenza A virus infections of humans (zoonotic transmission of avian or variant [swine-origin] influenza A viruses) occur sporadically.[65] Limited, non-sustained human-to-human transmission of some novel influenza A viruses has been reported following prolonged unprotected exposures to an ill index case.[66–68] Interspecies transmission and reassortment of influenza A viruses have been reported to occur among humans, pigs, and wild and domestic fowl. The influenza A viruses responsible for the 1918, 1957, 1968, and 2009

pandemics contained gene segments closely related to those of avian or swine influenza A viruses.[69–71] Control of influenza is a continuing human and veterinary public health concern.

Occupational Infections

LAIs, in the absence of animals, have not been well documented in the literature. However, it is believed that there is a risk of possible exposure to infectious influenza virus in the laboratory, especially through work with high concentrations of virus and/or experimental operations that generate aerosols (e.g., centrifugation, vortex-mixing). Animal-associated infections in the laboratory or the field have been reported.[72–74] LAIs may result from inoculation of mucous membranes including the upper respiratory tract through fomite transmission (e.g., touching virus-contaminated gloves to one's face following handling of tissues, feces, or secretions from infected animals; touching contaminated door handles or computer keyboards and then touching mucous membranes).

Natural Modes of Infection

Near-range inhalation through droplet/airborne spread is the predominant mode of influenza virus transmission among humans. Transmission may also theoretically occur through direct contact of contaminated surfaces and subsequent inoculation of mucous membranes including the upper respiratory tract since influenza viruses may persist for hours on surfaces particularly in the cold and under conditions of low humidity.[69] The incubation period is from one to four days. Recommendations for antiviral treatment and chemoprophylaxis of influenza are available.[75]

Laboratory Safety and Containment Recommendations

The agent may be present in respiratory tissues or secretions of humans and 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 the virus from aerosols generated by infecting animals or by aspirating, dispensing, mixing, centrifuging, or otherwise manipulating virus-infected materials. 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.

Seasonal Human Influenza Viruses BSL-2 facilities, practices, and procedures are recommended for diagnostic research and production activities utilizing contemporary influenza A, B, and C viruses circulating among humans (e.g., H1/H3/B). ABSL-2 is appropriate for work with these viruses in animal models.

Zoonotic and Animal Influenza A Viruses BSL-3 or ABSL-3 containment, with enhancements directed by regulatory authorities, should be used for laboratory work with low pathogenicity avian influenza (LPAI) A viruses that have caused zoonotic infections, particularly those with fatal outcomes (e.g., H7N4, H10N8). Work with Asian lineage A(H7N9) and non-U.S.LPAI A viruses should also be conducted in BSL-3 or ABSL-3 laboratories with practices, procedures, and facilities enhancements, as directed by regulatory authorities.

BSL-2 with enhanced facilities, practices, and procedures, as directed by regulatory authorities, should be used for working with domestic LPAI A viruses (e.g., H1–4, H6, H8–16) and equine, canine, and swine influenza A viruses. ABSL-2 with enhancements directed by regulatory authorities is appropriate for work with these viruses in animal models. Asian lineage A(H7N9) LPAI viruses have caused sporadic zoonotic infections with high mortality in humans since 2013.[76]

Non-Contemporary Human Influenza Viruses Non-contemporary, wild-type human influenza A(H2N2) viruses or reassortants containing the H2 or N2 RNA segments should be handled with increased caution. Important considerations in working with these viruses are the number of years since an antigenically related virus last circulated and the potential presence of a susceptible population. BSL-3 and ABSL-3 practices, procedures, and facilities are recommended with rigorous adherence to respiratory protection and clothing change protocols. Negative pressure, HEPA-filtered respirators and eye protection, or positive air-purifying respirators (PAPRs) are recommended for use. Cold-adapted, live attenuated A(H2N2) vaccine viruses may be worked with at BSL-2, but it is recommended that a risk assessment be performed before working with such viruses, and attention should be paid to prevent generation of reassortants that have H2 and/or N2 RNA segments and lack attenuating features of the parental attenuated viruses.

Historical, wild-type human influenza A(H1N1) and A(H3N2) viruses that have not circulated among humans in many years should be handled with increased precaution since younger adult workers and children have little or no immunity against such viruses. It is recommended that a risk assessment be performed before working with such viruses; this would include consideration of the number of years since a closely related virus last circulated among humans. For example, pre-2009 A(H1N1) viruses have not circulated in humans since the 2009–2010 season and there is little antigenic similarity between these viruses and the A(H1N1)pdm09 viruses that were responsible for the 2009 influenza pandemic. Other examples may arise in the future. In such cases, a more cautious approach to containment utilizing elevated Biosafety Levels and practices is warranted (e.g., BSL-2 with enhanced practices, procedures, and facilities).

1918 Influenza A(H1N1) Pandemic Virus Any research involving reverse genetics of the 1918 influenza A(H1N1) pandemic virus should proceed with extreme caution. Research findings suggest that exposure to A(H1N1)pdm09 virus through immunization or infection would provide protection against the reconstructed 1918 A(H1N1) virus.[77] Moreover, several serological studies of the A(H1N1)pdm09 virus have provided evidence for the presence of preexisting, cross-reactive antibodies to a 1918-like H1N1 virus from previous vaccinations or infections.[78,79] However, the 1918 A(H1N1) virus is still considered to pose both biosafety and biosecurity threats. The following practices and conditions are recommended for manipulation of reconstructed 1918 influenza A(H1N1) viruses and laboratory animals infected with the viruses. These following practices and procedures are considered minimum standards for work with the fully reconstructed virus.

- BSL-3 and ABSL-3 practices, procedures, and facilities;
- Animals, including non-human primates (NHPs), should be housed in primary barrier systems in ABSL-3 facilities;
- Use of negative pressure, HEPA-filtered respirators, or PAPRs;
- Rigorous adherence to respiratory protection and clothing change protocols;
- HEPA filtration for treatment of exhaust air; and
- Personal showers prior to exiting the laboratory.

Highly Pathogenic Avian Inluenza (HPAI) A Viruses Manipulating HPAI A viruses (e.g., H5, H7) in biomedical research laboratories also requires additional precautions because some viruses may pose increased risk to laboratory workers and have significant agricultural and economic implications. BSL-3 and ABSL-3 with enhanced practices, procedures, and facilities, as directed by regulatory authorities, are required, including clothing change and personal showering protocols. Loose-housed animals infected with HPAI A viruses must be contained within ABSL-3Ag facilities. See Appendix D for additional information. Negative pressure, HEPA-filtered respirators and eye protection, or positive air-purifying respirators are recommended for HPAI A viruses with potential to infect humans.

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 IBC, or equivalent resource, should consider but not limit consideration to the following in the conduct of protocol-driven risk assessment.

- The gene constellation used;
- Any mutations that are introduced and may result in enhancement of a pathogen's transmissibility and/or virulence;[80]
- 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; and
- 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 to containment, utilizing elevated Biosafety Levels and practices, is warranted.

Special Issues

Occupational Health Considerations Institutions performing work with HPAI and LPAI A viruses that have infected humans; non-contemporary wild-type human influenza A viruses, including recombinants and reassortants; and viruses created by reverse genetics of extinct virus strains (e.g., 1918 strain) should develop and implement a specific medical surveillance and response plan. At a minimum, these plans should: 1) strongly recommend annual vaccination with a currently licensed influenza vaccine for such individuals; 2) provide employee counseling regarding disease signs and symptoms including fever, conjunctivitis, and respiratory symptoms; 3) establish a protocol for monitoring personnel for these symptoms; 4) include collection of acute and convalescent serum samples in the event of a possible LAI; and 5) establish a clear medical protocol for responding to suspected Laboratory-associated infections. Antiviral drugs (e.g., oseltamivir, zanamivir) should be available for treatment of illness or post-exposure treatment/chemoprophylaxis, as necessary.[75] It is recommended that the virus under study be tested for susceptibility to antiviral drugs. All personnel should be enrolled in an appropriately constituted respiratory protection program.

Select Agent The reconstructed 1918 influenza A(H1N1) virus and HPAI viruses are Select Agents requiring registration with CDC or USDA for possession, use, storage, and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation and transfer of animal-origin viruses and diagnostic specimens obtained from animals require APHIS importation permits. CDC/PHS import permits are required for importation of seasonal influenza A, B, and C viruses and specimens obtained from humans. CDC/PHS permits may also be required for importation of animal-origin influenza viruses of known zoonotic potential. Importation and transfer of Select Agent viruses require APHIS/CDC importation permits. APHIS permit-driven containment, facility requirements, and personnel practices and/or restrictions may be applied for the possession and handling of animal-origin and zoonotic viruses. This may also include laboratory data/results to exclude the possibility of contamination with HPAI Select Agent viruses in specimens. A DoC export license or license exemption may be required for the export of Select Agent viruses 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 prototypical member of the family *Arenaviridae*.

Occupational Infections

LAIs with LCM virus are well documented. Most infections occur when chronic viral infection exists in laboratory or pet rodents, especially mice, hamsters, and guinea pigs.[81–83] Nude and severe combined immune deficient (SCID) mice may pose a special risk of harboring silent chronic infections. Mice shedding the virus may be asymptomatic. Inadvertently infected cell cultures also present a potential source of infection and dissemination of the agent.

Natural Modes of Infection

LCMV infections have been reported in Europe, the Americas, Australia, and Japan, and may occur wherever infected rodent hosts 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.[84]

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 by *Callitrichid* hepatitis virus, a strain of LCMV, have also occurred in NHPs in zoos, including macaques and marmosets.

Humans become infected by inhaling infectious aerosolized particles of rodent urine, feces, or saliva; by ingesting food contaminated with the virus; by contamination of mucous membranes with infected body fluids; or by directly exposing cuts or other open wounds to virus-infected blood. Several clusters of organ recipients from donors with unrecognized acute LCMV infection have been described with poor survival rates in the immunosuppressed recipients.[85–89] The source of donors' infection is usually untraceable except in one case where a pet hamster that was not overtly ill was incriminated.[89] Pregnant women infected with LCMV have transmitted the virus to their fetuses that resulted in death or serious central nervous system malformation as a consequence.[90]

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.[81]

Of special note, tumors may acquire LCMV as an adventitious virus without obvious effects on the tumor. The 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 occur.

Women of childbearing age should be made aware of risks posed by LCMV or rodents potentially infected by LCMV. Women who are pregnant or planning to become pregnant should be provided medical counseling that informs them of these risks with LCMV or animals potentially infected with LCMV.

Strains of LCMV that are shown to be lethal in non-human primates should be handled at BSL-3. BSL-3 is also 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. Work with infected hamsters should be done at ABSL-3.

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. ABSL-2 practices, containment equipment, and facilities are suitable for studies in adult mice with mouse brain-passaged strains requiring BSL-2 containment.

Special Issues

Vaccines Vaccines are not available for use in humans.

Transfer of Agent Importation of this agent requires 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*. Picornaviruses are small viruses with an RNA genome. Enteroviruses are likely transient inhabitants of the gastrointestinal tract and are stable at acid pH. There are three poliovirus serotypes: PV1, PV2, and PV3. Immunity to one serotype does not produce significant immunity to the other two.

Occupational Infections

Laboratory-associated poliomyelitis is uncommon. Twelve cases, including two deaths, were reported between 1941 and 1976.[91,92] Several instances of asymptomatic laboratory infections with poliovirus have been reported, but no laboratory-associated poliomyelitis has been reported for over 40 years. Both inactivated poliovirus vaccine (IPV) and oral poliovirus vaccine (OPV) are

highly effective in preventing disease. OPV alone induces mucosal immunity, which gradually fades over subsequent years. Poliovirus infections among immunized laboratory workers remain undetermined in the absence of laboratory confirmation. An immunized laboratory worker may unknowingly be a source of poliovirus transmission to susceptible persons in the community.[93] In April 2017, a spill of WPV2 in a production facility in the Netherlands infected one operator whose stool tested positive for poliovirus. This incident highlights the risk of containment breach and emphasizes the need for appropriate incident response planning and government oversight.[94]

Natural Modes of Infection

Humans are the only known reservoir of poliovirus, which is transmitted most frequently by persons with inapparent infections. Person-to-person spread of poliovirus via the fecal-oral route is the most common route of transmission, although the oral-oral route may account for some cases. Only one in several hundred infections of unimmunized persons with wild poliovirus leads to paralytic disease, with the vast majority of infections being asymptomatic or accompanied by minor, flu-like symptoms.

At one time, poliovirus infection occurred throughout the world. Transmission of wild poliovirus ceased in the United States by 1979. 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, led by the World Health Organization, has dramatically reduced the number of paralytic cases.

The last case of wild PV2 (WPV2) was detected in 1999, and certification of WPV2 eradication occurred in 2015. Since WPV2 was eradicated, all polio cases associated with PV2 have been caused by oral polio vaccine (OPV) directly (vaccine-associated paralytic polio [VAPP]) or by vaccine-derived polio type 2 virus (VDPV2). Due to continued occurrence of VAPP and outbreaks and chronic infections associated with VDPV2, WHO discontinued all routine OPV2 use as of May 1, 2016 by coordinating a global switch from trivalent OPV to bivalent OPV, containing only OPV1 and 3, along with the introduction of a single dose of inactivated polio vaccine (IPV). The last case of WPV3 occurred in Nigeria in 2012 and certification of WPV3 eradication occurred in 2019. As of 2019, only three countries (Pakistan, Afghanistan, and Nigeria) are considered to be endemic for WPV1. Complete polio eradication is expected in the near future.

Laboratory Safety and Containment Recommendations

Poliovirus is present in stool and in throat secretions of infected persons and in lymph nodes, brain tissue, and spinal cord tissue in fatal cases. In addition, poliovirus may be present in environmental samples (e.g., sewage).

Ingestion and parenteral inoculation are the primary routes of infection for laboratory workers. For immunized persons parenteral inoculation likely presents a lower risk. The potential for aerosol exposure is unknown. Laboratory animal-associated infections have not been reported, but infected non-human primates should be considered to present a risk.

Laboratory personnel working with and visitors with access to known poliovirus or infectious materials potentially containing poliovirus must have documented polio vaccination. Persons who have had a primary series of OPV or IPV and who are at an increased occupational risk should receive another dose of IPV. Available data do not indicate the need for more than a single lifetime IPV booster dose for adults.[95]

Type 2 and WPV3 Declaration of WPV2 eradication and the termination of routine OPV2 use initiated the containment of PV2 under the WHO Global Action Plan III (GAPIII).[96] GAPIII seeks to decrease the risk of reintroduction of eradicated polioviruses from laboratories and other facilities by calling for the destruction of non-essential poliovirus materials and containment of retained poliovirus material in certified poliovirus-essential facilities that adhere to the containment measures specified in GAP III. These measures include a biorisk management system, biosafety, security, and physical laboratory features and, at the time of this writing, apply to WPV2 and VDPV types 2 and 3, VDPV2, and OPV2 infectious materials as well as WPV and VDPV potentially infectious materials (e.g., fecal, respiratory secretion, and environmental samples collected at a time and in a place where WPV or VDPV was present). The U.S. National Authority for Containment (NAC) of Poliovirus at the CDC is responsible for working with poliovirus facilities to achieve certification. At the time of final eradication of all poliovirus types, additional GAPIII physical laboratory containment measures will be required for WPV and VDPV materials.

OPV2 potentially infectious materials are subject to the Guidance for non-poliovirus facilities to minimize risk of sample collections potentially infectious for polioviruses.[97,98] This document assigns risk categories based on the material and work performed and outlines specific risk mitigation measures that are much less stringent than GAPIII.

Type 1 and OPV3 When final eradication is declared, GAPIII containment will also apply to types 1 and OPV3. Laboratories and other facilities are encouraged to destroy all PV1 and OPV3 materials not essential for research or other work.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for all activities using poliovirus infectious and potentially infectious materials, including environmental and clinical samples. Contact the U.S. NAC for enhanced measures for work with eradicated poliovirus types and strains.

Laboratories should work with attenuated Sabin OPV strains unless there are strong scientific reasons for working with wild polioviruses. Contact the NAC for additional measures for work with WPV and VDPV types 2 and 3, and OPV2 infectious materials.

Special Issues

Transfer of Agent Importation of this agent requires 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. Contact the NAC prior to transfers of polioviruses.

Poxviruses

Four genera within the *Chordopoxvirinae* subfamily (family *Poxviridae*) contain species that can cause human disease: *Orthopoxvirus, Parapoxvirus, Yatapoxvirus,* and *Molluscipoxvirus*.[99] Most species in these genera are zoonotic with the exception of variola virus (*Orthopoxvirus*) and molluscum contagiosum virus (*Molluscipoxvirus*), which are solely human pathogens.[100,101] As most Laboratory-associated infections involve accidents associated with orthopoxviruses, only species of this genus will be discussed further.

Occupational Infections

Vaccinia virus is the prototypical orthopoxvirus, and its well-studied characteristics make it commonly used in both general and biomedical research.[102] Thus, vaccinia virus is the leading agent of laboratory-associated poxvirus infections. LAIs with replication-competent species, including wild-type and modified strains of vaccinia virus, have occurred even in previously vaccinated laboratorians. Other persons at risk for occupational exposure include animal care personnel having direct contact with vaccinated or infected animals or their secretions, or healthcare personnel who care for vaccinated or infected patients or administer a live vaccinia virus.[102,103]

The manifestation of infection is dependent upon factors such as virus species, route of entry, and host immune status. Infection results in the development of one to several lesions (localized) or a generalized rash (systemic) on the skin and/or mucous membranes. Infection with variola or monkeypox virus causes a febrile prodrome that is preceded by a distinct systemic rash illness. Vaccinia virus and cowpox virus typically cause a single lesion at the site of infection; however, multiple lesions and a generalized rash may also take place. Uncomplicated disease typically resolves within several weeks.[99,100]

Natural Modes of Infection

The most well-known orthopoxvirus is variola virus, which causes smallpox. After an extensive vaccination campaign, smallpox was declared eradicated in 1980. Monkeypox occurs sporadically in several West and Central African countries but remains endemic in the Democratic Republic of Congo. The importation of wild-caught animals from Ghana into the United States resulted in a 2003 monkeypox outbreak that affected multiple states. Vaccinia virus is used to make the current smallpox vaccine. Naturally-acquired infections with vaccinia virus exist outside of the United States.[104] Cases of human cowpox occur in Europe and Asia. Rodents are known or suspected to play a part in the transmission of monkeypox, cowpox, and vaccinia viruses.[99–101]

Laboratory Safety and Containment Recommendations

Vaccination with vaccinia virus can afford protection against infection from other species of orthopoxviruses. Smallpox vaccination occurs via scarification using a multi-puncture method with a bifurcated needle. The current U.S.-licensed smallpox vaccine, ACAM2000, uses a replication-competent vaccinia virus strain. Symptoms such as fever, headache, and swollen lymph nodes are prevalent following vaccination. Adverse events include localized reactions (e.g., robust take), unintentional transfer of virus (e.g., self-inoculation, ocular vaccinia), diffuse dermatologic complications (e.g., eczema vaccinatum, non-specific post-vaccination rash), progressive vaccinia, cardiac complications, fetal vaccinia, and postvaccinial central nervous system disease. Due to the severity of complications that can arise from vaccination, the vaccine is not recommended for persons with certain contraindications.[99,103,105,106]

Orthopoxviruses are stable in a wide range of environmental temperatures and humidity. Virus may enter the body through the mucous membranes (e.g., eye splashes, inhalation of droplets or fine-particle aerosols), broken skin (e.g., needlesticks, scalpel cut), ingestion, or by parenteral inoculation. Sources of exposure include fomites, infected human or animal tissue, excretions or respiratory secretions, or infectious cultures.[106]

Routine vaccination with ACAM2000 is recommended for laboratory personnel who directly handle cultures or animals contaminated or infected with replication-competent vaccinia virus, recombinant vaccinia viruses derived from replication-competent vaccinia strains (i.e., those that are capable of causing clinical infection and producing infectious virus in humans), or other orthopoxviruses that infect humans (e.g., monkeypox, cowpox, and variola).[106] Vaccination is advised every three years for work with monkeypox and variola viruses, and every 10 years for cowpox and vaccinia viruses. Vaccination is not required for individuals working in laboratories that only manipulate replication-deficient strains of vaccinia virus (modified virus Ankara [MVA], NYVAC, TROVAC,

and ALVAC). Vaccination may be offered to healthcare workers, animal care personnel, and vaccinators who have contact with contaminated materials. Vaccination does not protect against non-Orthopoxvirus species.[103,106]

Research with variola virus is restricted to two WHO-approved BSL-4 and ABSL-4 facilities; one is the CDC in Atlanta, GA, and the other is the State Research Center of Virology and Biotechnology (VECTOR) in Koltsovo, Russia. 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 laboratory work with monkeypox virus. BSL-2 and ABSL-2 containment plus vaccination are recommended for work with vaccinia and other human pathogenic poxviruses. The lowering of containment to BSL-1 for the manipulation of attenuated poxviruses and vectors (e.g., modified virus Ankara [MVA], NYVAC, TROVAC, and ALVAC) in areas where no other human orthopoxviruses are being used may be considered. 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. BSL-2 and ABSL-2 plus vaccination are recommended for work with most other poxviruses. Note that for research subject to the *NIH Guidelines*, approval to lower containment from BSL-2 must be requested from NIH Office of Science Policy.[107]

Special Issues

The CDC provides information on a variety of topics relating to variola, monkeypox, and vaccinia viruses online at https://www.cdc.gov. For non-emergency information on potential human infections, smallpox vaccination, or treatment options, the CDC Poxvirus Inquiry Line can be contacted at 404-639-4129 or CDC-Info can be reached at 800-232-4636. To obtain smallpox vaccine, CDC Drug Services can be reached by phone at 404-639-3670 or by email at drugservice@cdc.gov. Clinicians or health departments may contact the CDC Emergency Operations Center in critical circumstances.

Select Agent Congo Basin monkeypox, Variola major, and Variola minor are Select Agents requiring registration with CDC for possession, use, storage, and/or transfer. See Appendix F for additional information.

Transfer of Agent The importation of poxviruses into the United States and/or their interstate transport may be subject to the rules and regulations of the CDC Import Permit Program, CDC Division of Select Agents and Toxins, and/or the USDA Animal and Plant Health Inspection Service. The exportation of poxviruses may require a DoC permit.

Rabies Virus and related lyssaviruses

Rabies is an acute, progressive, fatal encephalitis caused by negative-stranded RNA viruses in the genus *Lyssavirus*, family *Rhabdoviridae*.[108,109] *Rabies lyssavirus* (formerly Rabies virus) is the representative member (type species) of the genus and is responsible for the majority of human and animal cases of rabies worldwide. Currently, there are 14 recognized viral species within the genus Lyssavirus, which can be found in Table 1.

Occupational Infections

Rabies LAIs are extremely rare; two cases have been documented. Both cases resulted from presumed exposure to high concentrations of infectious aerosols—one generated in a vaccine production facility[110] and the other in a research facility.[111] Naturally or experimentally-infected animals, their tissues, and their excretions are also a potential source of exposure for laboratory and animal care personnel.

Natural Modes of Infection

The natural hosts of rabies virus are many bat species and terrestrial carnivores, but any mammal 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, saliva, and lacrimal secretions, 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 needlesticks 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.[110,111]

Additional precautions (such as BSL-2 with BSL-3 practices) should be considered when working with lyssaviruses other than rabies virus; refer to Table 1. 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.[112] Rabies

vaccination is also recommended for all individuals entering or working in the same room where lyssaviruses or infected animals are used. The presence of virus-neutralizing antibodies in vaccinated individuals should be ascertained.[112,113] Prompt administration of post-exposure booster vaccinations is recommended following recognized exposures in previously vaccinated individuals per current guidelines.[112,113]

In cases where it is not possible to open the skull or remove the brain within a BSC, such as an autopsy or routine diagnostics, use appropriate methods and personal protective equipment (PPE), including dedicated laboratory clothing, heavy or chainmail gloves to avoid cuts or sticks from cutting instruments or bone fragments, and an N95 respirator combined with a face shield or a PAPR to protect the skin and mucous membranes of the eyes, nose, and mouth from exposure to tissue fragments or infectious droplets. Ample coverage of a 10% bleach solution should be used during and after the procedure for decontamination of exposed or contaminated surfaces and equipment.[114]

To prevent the generation of aerosols, a handsaw is recommended instead of an oscillating saw and contact of the saw with brain tissue is avoided. 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.

Table 1. Viruses currently included in the genus *Lyssavirus*

Species	Acronym	Recommended Biosafety Level
Aravan lyssavirus*	ARAV	2
Australian bat lyssavirus	ABLV	2
Bokeloh bat lyssavirus*	BBLV	2
Duvenhage lyssavirus	DUVV	2
European bat 1 lyssavirus	EBLV-1	2
European bat 2 lyssavirus	EBLV-2	2
Ikoma lyssavirus*	IKOV	3
Irkut lyssavirus	IRKV	2
Khujand lyssavirus*	KHUV	2
Lagos bat lyssavirus*	LBV	3
Mokola lyssavirus	MOKV	3
Rabies lyssavirus	RABV	2
Shimoni bat lyssavirus*	SHIBV	3
West Caucasian bat lyssavirus*	WCBV	3

*No human cases have been documented

Notes: This table is final as of publication, but it will be updated in future editions of BMBL to reflect the discovery of new, divergent lyssaviruses. When handled in a BSL-2 laboratory, BSL-3 pracitices and procedures should be used.

Special Issues

The CDC provides information on a variety of topics relating to Rabies virus, lyssaviruses, and pre/post-exposure prophylaxis online at https://www.cdc.gov. For non-emergency information on potential human infections, or treatment options, the CDC Rabies Duty Officer can be contacted at 404-639-1050 or CDC-Info can be reached at 800-232-4636.

Transfer of Agent Importation of this agent requires 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: 1) the *Orthoretrovirinae* with six genera including the genus *Lentivirus*, which includes HIV-1, HIV-2, and SIVs; the genus *Deltaretrovirus*, which includes human and simian T-lymphotropic viruses (HTLV-1, HTLV-2, HTLV-3, HTLV-4, and STLVs); and the genus *Betaretrovirus*, which includes simian type D retrovirus (SRV); and 2) the *Spumaretrovirinae*, which has recently been updated to contain five genera,[115] including the genus *Simiispumavirus*, which includes simian foamy viruses (SFVs) that can occasionally infect humans in close contact with infected non-human primates (NHPs). Of these, only HIV and HTLV are pathogenic in humans and are now classified as known human carcinogens in the National Toxicology Program's Report on Carcinogens.[53] SIV/HIV genetic recombinants, known as SHIVs, are used in NHPs as models of HIV infection. The composition of SHIVs can vary but generally consist of an SIV genetic backbone containing specific HIV genes or gene regions.

Occupational Infections

Since 1991, data on occupational HIV transmission in health care workers (HCW) have been collected through a CDC-supported National HIV Surveillance system following a standardized case investigation protocol by state health department HIV staff with help from CDC.[116,117] 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 this system are classified as either documented or possible occupational transmission. Those classified as documented occupational transmission had evidence of HIV seroconversion (i.e., a negative HIV-antibody test at the time of the exposure that converted to positive) following a discrete percutaneous or mucocutaneous occupational exposure to blood, body fluids, or other clinical or laboratory specimens. As of 2013, confirmed HIV infections among 58 HCWs were reported, including 20 laboratory workers, of

which only one involved a laboratory worker who sustained a needle exposure while working with an HIV-infected culture. There were another 49 HCWs exposed to HIV-infected blood, including four persons exposed to concentrated virus in a laboratory.[116,117]

Workers have been reported to develop antibodies to simian immunodeficiency virus (SIV) following exposures.[118–120] One case was associated with a needlestick that occurred while the worker was manipulating a contaminated needle after bleeding an SIV-infected macaque monkey.[121] 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.[118] A third worker was exposed to SIV-infected primate blood through a needlestick and subsequently developed antibodies to SIV.[118] Of these three persons, only the worker exposed via dermatitis showed evidence of a persistent infection. To date, there is no evidence of illness or immunological incompetence in any of these workers. However, workers who have been occupationally exposed to HIV/SIV are recommended to immediately start an antiretroviral regimen. SFV infections in humans have occurred due to cross-species transmission following a variety of NHP exposures (e.g., working with NHPs, hunting and butchering NHPs) resulting in life-long, persistent infection but without any evidence for disease. Higher prevalences have been reported in individuals exposed to NHPs by bites, especially those reporting severe bite wounds. There has been a report of a laboratory infection while handling SFV.[119] Laboratory infection with SRV has been reported in two workers but without molecular evidence of persistent infection or disease.[122] SRV infection was also reported in one AIDS patient with lymphoma but without a history of NHP contact. Dual infection of a laboratory worker with SFV and SRV has also been reported but without evidence of secondary transmission of disease.[122] STLV infection of laboratory workers has not been reported but is known to occur in persons who hunt NHPs.[123,124]

Natural Modes of Infection

Retroviruses are widely distributed as infectious agents of vertebrates, including NHPs. Within the human population, the spread of HIV and HTLV is by close sexual contact, parenteral exposure through blood, blood-derived products, or other potentially infectious materials and from mother to child. Transmission of SFV and SRV from infected persons has not been reported.[122,124,125]

SIV infection of NHPs rarely causes disease but can lead to immunodeficiency and AIDS-like illness similar to that seen in HIV-infected humans.[123] STLV infection of NHPs has been reported to cause T-cell lymphomas and leukemia, generalized skin lesions, and splenomegaly.[123] SRV-infected macaques can show symptoms similar to AIDS in humans, and this presentation is called simian AIDS (SAIDS).[123] SRV-infected macaques have also displayed retroperitoneal

fibromatosis, necrotizing stomatitis with osteomyelitis, acute death, splenomegaly, lymphadenopathy, and fibroproliferative disorders. Disease has not been associated with NHPs naturally infected with SFV.[123]

Laboratory Safety and Containment Recommendations

HIV and HTLV have been isolated from blood, semen, saliva, urine, CSF, amniotic fluid, breast milk, cervical secretions, and tissues of infected persons and experimentally infected NHPs. Additionally, HIV has been isolated from tears of infected persons.

SIV, SHIV, and STLV have been isolated from blood, CSF, and a variety of tissues of infected NHPs.[123] 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 primate-derived tissue cultures, in animals experimentally infected or inoculated with SIV, SHIV, or STLV, in all materials derived from SIV, SHIV, and STLV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.[126]

SFV and SRV have been isolated from NHP blood and a variety of other tissues and can be cultured in vitro. Virus should be presumed to be present in all NHP-derived tissue cultures, in animals experimentally infected or inoculated with SFV or SRV, in all materials derived from SFV or SRV cultures, and in/on all equipment and devices coming into direct contact with any of these materials, similar to the handling of human clinical materials.[123]

Although the risk of occupationally-acquired infection with retroviruses 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.

In the laboratory, retroviruses 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 retrovirus cultures, in all materials derived from retrovirus cultures, and in/on all equipment and devices coming into direct contact with any of these materials.

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.

Activities involving large-scale volumes or preparation of concentrated retroviruses, including HIV, SIV, or SHIV, should be conducted at BSL-3. Activities, such as producing research-laboratory-scale quantities of retroviruses, including HIV, SIV or SHIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, can be performed in a BSL-2 facility using BSL-3 practices.

Standard Precautions and personal protective equipment should be used when working with all body fluids even if the infection status of the individual or animal is unknown.[126] BSL-2 practices, containment equipment, and facilities are recommended for activities involving blood-contaminated clinical specimens, body fluids, and tissues from NHPs and humans infected with retroviruses. ABSL-2 is appropriate for NHPs and other animals infected with retroviruses, including HIV, SIV, or SHIV. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BSL-2. Since 1996, post-exposure prophylaxis with antiretrovirals has been recommended to prevent infection following occupational exposures.[127]

In addition to the aforementioned recommendations, persons working with any retrovirus, including HIV, SIV, or SHIV, or other bloodborne pathogens, should consult the OSHA Bloodborne Pathogen Standard.[43]

Special Issues

It is recommended that all institutions establish written policies (e.g., treatment, prophylaxis protocols) regarding the management of laboratory exposure to retroviruses (HIV, SIV). See Section VII for additional information.

The risk associated with retroviral vector systems can vary significantly, especially lentiviral vectors. Because the risk associated with each gene transfer system can vary, it is recommended that all gene transfer protocols be reviewed by the institution's biosafety review committee or IBC.

Transfer of Agent Importation of this agent or materials containing 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) and Middle East Respiratory Syndrome (MERS) Coronaviruses

Note: the 6th edition of the BMBL had already undergone final clearance at the time of the 2019 coronavirus pandemic. For the latest biosafety recommendations regarding work with SARS Coronavirus 2 (SARS-CoV-2) please consult the CDC COVID-19 website at (https://www.cdc.gov/coronavirus/2019-nCoV/index.html).

Several human coronaviruses have been identified that can be broadly classified into low and high pathogenicity. Low pathogenic human coronaviruses include 229E, HKU1, OC43, and NL63. High pathogenic coronaviruses include SARS and MERS-CoV. SARS is a viral respiratory illness caused by 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 Southeast Asian countries, North America, South America, and Europe following major airline routes.[128] 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.

In general, SARS patients present with fever (temperature greater than 100.4°F [>38.0°C]), malaise, and myalgia quickly followed by respiratory symptoms including shortness of breath and cough. Ten to 20% 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%.

A second human coronavirus that causes severe disease, Middle East Respiratory Syndrome coronavirus (MERS-CoV), was first identified in Saudi Arabia in September 2012.[128–130] Between 2012 and mid-2017, the WHO confirmed 1,952 cases with 693 deaths.[131] Cases have been confirmed in 27 countries, though all cases have been linked to residents of the Arabian Peninsula.[131] A wide clinical spectrum of MERS-CoV infections has been reported with asymptomatic infection identified during outbreaks, acute respiratory illness in most symptomatic patients, or severe presentation including rapidly progressive pneumonitis, respiratory failure, septic shock, or multi-organ failure resulting in death.[132] Globally, 35–40% of cases reported to WHO have resulted in fatality. Common signs and symptoms at hospital admission include fever, chills/rigors, headache, non-productive cough, dyspnea, and myalgia.

Occupational Infections

Three different episodes of SARS-CoV transmission to laboratory workers occurred in 2003 and 2004 in research laboratories in Singapore, Taiwan, and Beijing.[133–135] The events in 2004 involved two different laboratory personnel, with one case resulting in secondary and tertiary transmission of the virus to close contacts and healthcare providers.[133] Each occurrence was linked to a deviation from protocol or established laboratory practices.[134,135] Additionally, no laboratory-associated cases have been associated with the routine processing of SARS or MERS diagnostic specimens for detection of virus; however, both coronaviruses represent an emerging infectious disease for which risk to the medical and laboratory community is not fully understood; therefore, caution

should be exercised when handling specimens that could potentially contain SARS or MERS-CoV.

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 suspected or probable case.[136] SARS is thought to be spread primarily through droplets, aerosols, and possibly fomites. The natural reservoir for SARS-CoV is unknown.

MERS-CoV transmission can occur in hospital settings through close contact. In the community, transmission can occur between ill people and others through close contact. Transmission may also occur in the community through close contact with infected dromedary camels who may be a reservoir for the virus. The incubation period of MERS-CoV is usually two to five days; however, it can range from two to 14 days.[131]

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

Laboratory Safety and Containment Recommendations

SARS and MERS coronaviruses may be detected in respiratory, blood, urine, or stool specimens. The exact mode of transmission of coronavirus Laboratory-associated infections have not been established, but in clinical settings, the primary mode of transmission appears to be through direct or indirect contact of mucous membranes with infectious respiratory droplets.[136,137]

SARS and MERS coronavirus propagation in cell culture and the initial characterization of viral agents recovered in cultures of clinical specimens must be performed at BSL-3. Respiratory protection should be used by all personnel.

Inoculation of animals for potential recovery of SARS- or MERS-CoV for characterization of putative SARS or MERS agents must be performed in ABSL-3 facilities using ABSL-3 work practices. Respiratory protection should be used.

Activities involving manipulation of untreated specimens should be performed in BSL-2 facilities using 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 should be used.

In clinical laboratories, respiratory specimens, whole blood, serum, plasma, and urine specimens should be handled using Standard Precautions at BSL-2.[138] Work using intact, full-length genomic RNA should be conducted at BSL-2.

In the event of any break in laboratory procedure or accident (e.g., accidental spillage of material suspected of containing SARS- or MERS-CoV), procedures for emergency exposure management and 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 Personnel working with the virus or samples containing or potentially containing the virus should be trained regarding the symptoms of SARS- and MERS-CoV infection and counseled to report any fever or respiratory symptoms to their supervisor immediately. Post-exposure baseline serum samples should be taken following any potential exposures. Personnel should be evaluated for possible exposure and the clinical features and course of their illness should be closely monitored for any signs or symptoms of disease. Institutions performing work with SARS- or MERS-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:

- Deviation from protocol or established 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 are available from the CDC.[139] Laboratory workers who are believed to have had a laboratory exposure to SARS- or MERS-CoV should be evaluated, counseled about the risk of SARS- and MERS-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.

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

Transfer of Agent The importation of SARS- and MERS-CoV into the United States and/or its interstate transport may be subject to the rules and regulations of the CDC Import Permit Program, CDC Division of Select Agents and Toxins, and/or the USDA Animal and Plant Health Inspection Service. The exportation of SARS-CoV may require a DoC permit.

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Section VIII-F: Arboviruses and Related Zoonotic Viruses

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

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

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

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

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

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

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

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

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

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

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

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

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

Risk Group 2 Viruses with BSL-2 Containment Recommended

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

Laboratory Safety and Containment Recommendations

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

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

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

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

Risk Group 3 Viruses with BSL-3 Containment Recommended

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

Laboratory Safety and Containment Recommendations

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

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

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

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

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

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

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

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

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

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

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

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

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

Enhanced BSL-3 Containment

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

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

Risk Group 4 Viruses with BSL-4 Containment Recommended

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

Laboratory Safety and Containment Recommendations

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

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

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

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

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

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

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

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

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

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

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

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

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

West Nile Virus (WNV)

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

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

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

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

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

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

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

Occupational Infections

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

Natural Modes of Infection

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

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

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

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

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

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

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

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

Rift Valley Fever Virus (RVFV)

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

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

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

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

Occupational Infections

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

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

Natural Modes of Infection

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

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

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

Laboratory Safety and Containment Recommendations

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

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

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

Special Issues

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Section VIII-G: Toxin Agents

Botulinum Neurotoxin

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

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

Diagnosis of Laboratory Exposures

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

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

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

Laboratory Safety and Containment Recommendations

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

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

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

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

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

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

Special Issues

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

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

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

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

Staphylococcal Enterotoxins (SE)

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

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

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

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

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

Diagnosis of Laboratory Exposures

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

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

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

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

Laboratory Safety and Containment Recommendations

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

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

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

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

Special Issues

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

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

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

Ricin

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

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

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

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

Diagnosis of Laboratory Exposures

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

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

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

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

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

Laboratory Safety and Containment Recommendations

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

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

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

Special Issues

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

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

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

Selected Low Molecular Weight (LMW) Toxins

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

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

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

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

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

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

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

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

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

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

Diagnosis of Laboratory Exposures

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

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

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

Laboratory Safety and Containment Recommendations

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

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

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

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

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

Special Issues

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

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

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

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Section VIII-H: Prion Diseases

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

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

Occupational Infections

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

Modes of Infection and Spread

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

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

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

Table 1. Human Prion Diseases

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

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

Table 2. Animal Prion Diseases

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

Laboratory Safety and Containment Recommendations

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

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

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

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

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

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

Special Issues

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

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

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

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

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

Bovine Spongiform Encephalopathy

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

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

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

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

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

Table 3. Tissue Preparation for Human CJD and Related Diseases

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

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

Handling and processing of tissues from patients with suspected prion disease

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

Handling and processing of multiple human prion tissue samples

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

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

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

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

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

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

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

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

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

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

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