Section VIII—Agent Summary Statements

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 of 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 until the return of more favorable conditions. Reports of suspected anthrax outbreaks date back to as early as 1250 BC. The study of anthrax and *B. anthracis* in the 1800s contributed greatly to our general understanding of infectious diseases. Much of Koch's postulates were derived from work on identifying the etiologic agent of anthrax. Louis Pasteur developed the first attenuated live vaccine for anthrax.

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. Anthrax still occurs frequently in parts of central Asia and Africa. In the United States, it occurs sporadically in animals in parts of the West, Midwest and Southwest.

The infectious dose varies greatly from species to species and is route-dependent. The inhalation anthrax infectious dose (ID) for humans primarily has been extrapolated from inhalation challenges of nonhuman primates (NHP) or studies done in contaminated mills. Estimates vary greatly but the medium lethal dose (LD_{50}) is likely within the range of 2,500-55,000 spores.[1] It is believed that very few spores (10 or less) are required for cutaneous anthrax.[2]

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. Numerous cases of laboratory-associated anthrax (primarily cutaneous) have been reported.[3,4] Recent cases include suspected cutaneous anthrax in a laboratory worker in Texas and a cutaneous case in a North Dakota male who disposed of five cows that died of anthrax.[5,6]

Natural Modes of Infection

The clinical forms of anthrax in humans that result from different routes of infection are: 1) cutaneous (via broken skin); 2) gastrointestinal (via ingestion); and 3) inhalation anthrax. Cutaneous anthrax is the most common and readily treatable form of the disease. Inhalation anthrax used to be known as "Woolsorter disease" due to its prevalence in textile mill workers handling wool and other contaminated animal products. While naturally occurring disease is no longer a

significant public health problem in the United States, anthrax 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.[7]

Laboratory Safety and Containment Recommendations

B. anthracis may be present in blood, skin lesion exudates, cerebrospinal fluid, pleural fluid, sputum, and rarely, in urine and feces. The 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. Efforts should be made to avoid production of aerosols by working with infectious organisms in a BSC. In addition, all centrifugation should be done using aerosol-tight rotors that are opened within the BSC after each run.

BSL-2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures. ABSL-2 practices, containment equipment and facilities are recommended for studies utilizing experimentally infected laboratory rodents. BSL-3 practices, containment equipment, and facilities are recommended for work involving production quantities or high concentrations of cultures, screening environmental samples (especially powders) from anthrax-contaminated locations, and for activities with a high potential for aerosol production. Workers who frequently centrifuge *B. anthracis* suspensions should use autoclavable aerosol-tight rotors. In addition, regular routine swabbing specimens for culture should be routinely obtained inside the rotor and rotor lid and, if contaminated, rotors should be autoclaved before re-use.

Special Issues

Vaccines A licensed vaccine for anthrax is available. Guidelines for its use in occupational settings are available from the ACIP.[8,9] Worker vaccination is recommended for activities that present an increased risk for repeated exposures to *B. anthracis* spores including: 1) work involving production quantities with a high potential for aerosol production; 2) handling environmental specimens, especially powders associated with anthrax investigations; 3) performing confirmatory testing for *B. anthracis*, with purified cultures; 4) making repeated entries into known *B. anthracis*-spore-contaminated areas after a terrorist attack; 5) work in other settings in which repeated exposure to aerosolized *B. anthracis* spores might occur. Vaccination is not recommended for workers involved in routine processing of clinical specimens or environmental swabs in general diagnostic laboratories.

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

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A 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 highly specialized culture and transport media for cultivation in the laboratory. Its natural habitat is the human respiratory tract.

Occupational Infections

Occupational transmission of pertussis has been reported, primarily among healthcare workers.[10-16] Outbreaks, including secondary transmission, among workers have been documented in hospitals, long-term care institutions, and laboratories. Nosocomial transmissions have been reported in healthcare settings. Laboratory-acquired pertussis has been documented.[17,18]

Natural Modes of Infection

Pertussis is highly communicable, with person-to-person transmission occurring via aerosolized respiratory secretions containing the organism. The attack rate among susceptible hosts is affected by the frequency, proximity, and time of exposure to infected individuals. Although the number of reported pertussis cases declined by over 99% following the introduction of vaccination programs in the 1940s, the 3- to 4-year cycles of cases have continued into the post-vaccination era.[19-21]

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 in its infrequent manifestation of septicemia and pneumonia, respectively.[22,23] Because the natural mode of transmission is via the respiratory route, aerosol generation during the manipulation of cultures and contaminated clinical specimens generates the greatest potential hazard.

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 should be employed for housing experimentally infected animals. Primary containment devices and equipment, including biological safety cabinets, safety centrifuge cups or safety centrifuges should be used for activities likely to

generate potentially infectious aerosols. BSL-3 practices, containment equipment, and facilities are appropriate for production operations.

Special Issues

Vaccines Pertussis vaccines are available but are not currently approved or recommended for use in persons over six years of age. Because this recommendation may change in the near future, the reader is advised to review the current recommendations of the ACIP published in the Morbidity and Mortality Weekly Report (MMWR) and at the CDC Vaccines and Immunizations Web site for the latest recommendations for adolescents and adults.

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

Brucella species

The genus *Brucella* consists of slow-growing, very small gram-negative coccobacilli whose natural hosts are mammals. Seven *Brucella* species have been described using epidemiologic and biological characteristics, although at the genetic level all brucellae are closely related. *B. melitensis* (natural host: sheep/goats), *B suis* (natural host: swine), *B. abortus* (natural host: cattle), *B. canis* (natural host: dogs), and *B.* "*maris*" (natural host: marine mammals) have caused illness in humans exposed to the organism including laboratory personnel.[24,25] Hypersensitivity to *Brucella* antigens is a potential but rare hazard to laboratory personnel. Occasional hypersensitivity reactions to *Brucella* antigens occur in workers exposed to experimentally and naturally infected animals or their tissues.

Occupational Infections

Brucellosis has been one of the most frequently reported laboratory infections in the past and cases continue to occur.[4,26-28] Airborne and mucocutaneous exposures can produce LAI. Accidental self-inoculation with vaccine strains is an occupational hazard for veterinarians.

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 brucellae. Multiple routes of transmission have been identified, including direct contact with infected animal tissues or products, ingestion of contaminated milk, and airborne exposure in pens and stables.

Laboratory Safety and Containment Recommendations

Brucella infects the blood and a wide variety of body tissues, including cerebral spinal fluid, semen, pulmonary excretions, placenta, and occasionally urine. Most laboratory-associated cases occur in research facilities and involve exposures to *Brucella* organisms grown in large quantities or exposure to placental tissues containing *Brucella*. Cases have occurred in clinical laboratory settings from sniffing bacteriological cultures[29] or working on open bench tops.[30] Aerosols from, or direct skin contact with, cultures or with infectious clinical specimens from animals (e.g., blood, body fluids, tissues) are commonly implicated in human infections. Aerosols generated during laboratory procedures have caused multiple cases per exposure.[30,31] Mouth pipetting, accidental parenteral inoculations, and sprays into eyes, nose and mouth result in infection. The infectious dose of *Brucella* is 10-100 organisms by aerosol route and subcutaneous route in laboratory animals.[32,33]

BSL-2 practices, containment equipment, and facilities are recommended for routine clinical specimens of human or animal origin. Products of conception containing or believed to contain pathogenic *Brucella* should be handled with BSL-3 practices due to the high concentration of organisms per gram of tissue. BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended, for all manipulations of cultures of pathogenic *Brucella* spp. listed in this summary, and for experimental animal studies.

Special Issues

Vaccines Human *Brucella* vaccines have been developed and tested in other countries with limited success. A human vaccine is not available in the United States.[34]

Select Agent *Brucella abortus, Brucella melitensis, and Brucella 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 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.

Burkholderia mallei

Burkholderia mallei (formerly *Pseudomonas mallei*) is a non-motile gram-negative rod associated with glanders, a rare disease of equine species and humans. While endemic foci of infection exist in some areas of the world, glanders due to natural infection is extremely rare in he United States.

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.[35] Modes of transmission may include inhalation and/or mucocutaneous exposure.

Natural Mode of Infection

Glanders is a highly communicable disease of horses, goats, and donkeys. Zoonotic transmission occurs to humans, but person-to-person transmission is rare. Clinical glanders no longer occurs in the Western Hemisphere or in most other areas of the world, although enzootic foci are thought to exist in Asia and the eastern Mediterranean.[36] Clinical infections in humans are characterized by tissue abscesses and tend to be very serious.

Laboratory Safety and Containment Recommendations

B. mallei can be very hazardous in a laboratory setting. In a pre-biosafety era report, one-half of the workers in a *B. mallei* research laboratory were infected within a year of working with the organism.[37] Laboratory-acquired infections have resulted from aerosol and cutaneous exposure.[37,38] Laboratory infections usually are caused by exposure to bacterial cultures rather than to clinical specimens. Workers should take precautions to avoid exposure to aerosols from bacterial cultures, and to tissues and purulent drainage from victims of this disease.

Primary isolations from patient fluids or tissues may be performed with BSL-2 practices, containment equipment, and facilities in a BSC. Procedures must be performed under BSL-3 containment whenever infectious aerosols or droplets are generated, such as during centrifugation or handling infected animals, or when large quantities of the agent are produced. Procedures conducted outside of a BSC (centrifugation, animal manipulation, etc.) that generate infectious aerosols require respiratory protection. Sealed cups should be used with all centrifuges and these should be opened only inside a BSC. Gloves should be worn when working with potentially infectious material or animals. Animal work with *B. mallei* should be done with ABSL-3 practices, containment equipment, and facilities.

Special Issues

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 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.

Burkholderia pseudomallei

Burkholderia pseudomallei (formerly *Pseudomonas 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, Central America and South America. This organism is the causative agent of melioidosis, an unusual bacterial disease characterized by abscesses in tissues and organs. Victims of the disease frequently exhibit recrudescence months or years after the initial infection.

Occupational Infections

Melioidosis is generally considered to be a disease associated with agriculture; however, *B. pseudomallei* can be hazardous for laboratory workers. There are two reports of melioidosis in laboratory workers who were infected by aerosols or via skin exposure.[39,40] Laboratory workers with diabetes are at increased risk of contracting melioidosis.[41]

Natural Modes of Infection

While primarily a disease found in Southeast Asia and Northern Australia, melioidosis can occasionally be found in the Americas.[42] Natural modes of transmission include the exposure of mucous membranes or damaged skin to soil or water containing the organism, the aspiration or ingestion of contaminated water, or the inhalation of dust from contaminated soil. In endemic areas, 5-20% of agricultural workers have antibody titers to *B. pseudomallei*, in the absence of overt disease.[43]

Laboratory Safety and Containment Recommendations

B. pseudomallei can cause a systemic disease in human patients. Infected tissues and purulent drainage from cutaneous or tissue abscesses can be sources of infection. Blood and sputum also are potential sources of infection.

Work with clinical specimens from patients suspected of having melioidosis and of *B. pseudomallei* cultures may be performed with BSL-2 practices, containment equipment, and facilities. Work should be done in a BSC. Gloves always should be worn when manipulating the microorganism. In cases where infectious aerosols or droplets could be produced, or where production quantities of the organism are generated, these procedures should be confined to BSL-3 facilities with all pertinent primary containment against escape of aerosols. Respiratory protection must be used if the microorganism is manipulated outside of a BSC, such as during centrifugation or handling infected animals. Sealed

cups should be used in all centrifuges and these should be opened only in a BSC. Animal studies with this agent should be done at ABSL-3.

Special Issues

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

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

Campylobacter (C. jejuni subsp. jejuni, C. coli, C. fetus subsp. fetus, C. upsaliensis)

Campylobacters are curved, S-shaped, or spiral rods associated with gastrointestinal infections (primarily *C. jejuni* subsp. *jejuni* and *C. coli*), bacteremia, and sepsis (primarily *C. fetus* subsp. *fetus* and *C. upsaliensis*). Organisms are isolated from stool specimens using selective media, reduced oxygen tension, and elevated incubation temperature (43°C).

Occupational Infections

These organisms rarely cause LAI, although laboratory-associated cases have been documented.[44-47] Experimentally infected animals also are a potential source of infection.[48]

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 500-800 organisms has caused symptomatic infection.[49-51] Natural transmission usually occurs from ingestion of organisms in contaminated food or water and from direct contact with infected pets, farm animals, or infants.[52]

Laboratory Safety and Containment Recommendations

Pathogenic *Campylobacter sp.* may occur in fecal specimens in large numbers. *C. fetus* subsp. *fetus* may also be present in blood, exudates from abscesses, tissues, and sputa. The primary laboratory hazards are ingestion and parenteral inoculation of *C. jejuni*. 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 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.

Chlamydia psittaci (Chlamydophila psittaci), C. trachomatis, C. pneumoniae (Chlamydophila pneumoniae)

Chlamydia psittaci, *C. pneumoniae* (sometimes called *Chlamydophila psittaci* and *Chlamydophila pneumoniae*) and *C. trachomatis* are the three species of *Chlamydia* known to infect humans. Chlamydiae are nonmotile, gram-negative 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 birds and is highly pathogenic for humans. *C. trachomatis* is historically considered an exclusively human pathogen and is the most commonly reported bacterial infection in the United States. *C. pneumoniae* is considered the least pathogenic species, often resulting in subclinical or asymptomatic infections in both animals and humans.

Occupational Infections

Chlamydial infections caused by *C. psittaci* and *C. trachomatis* lymphogranuloma venereum (LGV) strains were at one time among the most commonly reported laboratory-associated bacterial infections.[26] In cases reported before 1955[4], the majority of infections were psittacosis, and these had the highest case fatality rate of laboratory-acquired infectious agents. The major sources of laboratory-associated psittacosis are contact with and exposure to infectious aerosols in the handling, care, or necropsy of naturally or experimentally infected birds. Infected mice and eggs also are important sources of *C. psittaci*. Most reports of laboratory-acquired 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.[53] Seroconversion to chlamydial antigens is common and often striking although early antibiotic treatment may prevent an antibody response.

Laboratory-associated infections with *C. pneumoniae* have been reported.[54] Exposed workers were asymptomatic and infection was diagnosed by serology. The route of infection was attributed to inhalation of droplet aerosols created during procedures associated with culture and harvest of the agent from cell culture.

With all species of *Chlamydia*, mucosal tissues in the eyes, nose, and respiratory tract are most often affected by occupational exposures that can lead to infection.

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, and neurologic complications. Natural infections are acquired by inhaling dried secretions from infected birds. Psittacine birds commonly kept as pets (parrots, parakeets, cockatiels, etc.) 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 a body of evidence associating this agent with chronic diseases such as atherosclerosis and asthma.

Laboratory Safety and Containment Recommendations

C. psittaci may be present in the tissues, feces, nasal secretions and blood of infected birds, and in blood, sputum, and tissues of infected humans. *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*. 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 centrifuge malfunctions, also pose a risk for infection.

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 (A through K) of *C. trachomatis* or *C. pneumoniae*.

BSL-3 practices, containment equipment, and facilities are 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. Activities involving non-avian strains of *C. psittaci* may be performed in a BSL-2 facility as long as BSL-3 practices are followed, including but not limited to the use of primary containment equipment such as BSCs. ABSL-3 practices, containment equipment, and facilities and respiratory protection are recommended for personnel working with naturally or experimentally infected caged birds.

BSL-3 practices and containment equipment are recommended for activities involving work with culture specimens or clinical isolates known to contain or be potentially infected with the LGV serovars (L_1 through L_3) of *C. trachomatis*. 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, including but not limited to use of primary containment equipment such as BSCs.

Gloves are recommended for the necropsy of birds and mice, the opening of inoculated eggs, and when there is the likelihood of direct skin contact with infected tissues, bubo fluids, and other clinical materials.

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*.

BSL-3 practices, containment equipment, and facilities are indicated for activities involving any of these species with high potential for droplet or aerosol production and for activities involving large quantities or concentrations of infectious materials.

Special Issues

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

Neurotoxin-producing Clostridia species

Clostridium botulinum, and rare strains of *C. baratii* and *C. butyricum,* are anaerobic spore-forming species that cause botulism, a life-threatening food-borne illness. The pathogenicity of these organisms results from the production of botulinum toxin, one of the most highly potent neurotoxins currently recognized. Purified botulinum neurotoxin is a 150 kDa protein that acts selectively on peripheral cholinergic nerve endings to block neurotransmitter release.[55] The principal site of action is the neuromuscular junction, where blockade of transmission produces muscle weakness or paralysis. The toxin also acts on autonomic nerve endings where blockade of transmission can produce a variety of adverse effects. The toxin may also contain associated proteins that may increase its size to as high as 900 kDA.

Occupational Infections

There has been only one report of botulism associated with handling of the toxin in a laboratory setting.[56] However, concerns about potential use of the toxin as an agent of bioterrorism or biological warfare have led to increased handling of the substance by investigators studying mechanism of action and/or developing countermeasures to poisoning.[57]

Natural Modes of Infection

Botulinum toxin occurs in seven different serotypes (A to G), but almost all naturally-occurring human illness is due to serotypes A, B, E, and F.[58] Botulism occurs when botulinum toxin is released into circulation following ingestion of preformed toxin. However, animal studies have shown that botulism may occur through inhalation of preformed toxin. Use of appropriate personal protective equipment should prevent potential exposure through mucus membranes. Symptoms and even death are possible by accidental injection of botulinum toxin. Risk to toxin exposure is dependent on both route of exposure and toxin molecular weight size. Exposure to neurotoxin producing Clostridia species does not cause infection; however, in certain rare circumstances (Infant Botulism, Wound Botulism, and Adult colonization), the organism can colonize the intestinal tract and other sites and produce toxin. In Wound Botulism, exposure to toxin is caused by introduction of spores into puncture wounds and *in situ* production by the organism. Infants less than 1 year of age may be susceptible to intestinal colonization and develop the syndrome of Infant Botulism as a result of *in situ* production of toxin. Similarly to Infant Botulism, ingestion of spores by adults with a compromised gastrointestinal tract (GI), such as following GI surgery or long-term administration of antibiotics, may increase risk for intestinal infection and *in situ* production of toxin. See the *C. botulinum* Toxin Agent Summary Statement and Appendix I for additional information.

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).[59] In addition, bacterial cultures may produce very high levels of toxin.[60] In healthy adults, it is typically the toxin and not the organism that causes disease. Risk of laboratory exposure is due to the presence of the toxin and not due to a potential of infection from the organisms that produce the toxin. Although spore-forming, there is no known risk to spore exposure except for the potential for the presence of residual toxin associated with pure spore preparations. Laboratory safety protocols should be developed with the focus on prevention of accidental exposure to the toxin produced by these *Clostridia* species.

BSL-2 practices, containment equipment, and facilities are recommended for activities that involve the organism or the toxin[61] including the handling of potentially contaminated food. Solutions of sodium hypochlorite (0.1%) or sodium hydroxide (0.1N) readily inactivate the toxin and are recommended for decontamination of work surfaces and for spills. Autoclaving of contaminated materials also is appropriate.

Additional primary containment and personnel precautions, such as those recommended for BSL-3, should be implemented for activities with a high potential for aerosol or droplet production, or for those requiring routine handling of larger quantities of the organism or of the toxin. ABSL-2 practices, containment equipment, and facilities are recommended for diagnostic studies and titration of toxin.

Special Issues

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

Post-Exposure Treatment An equine antitoxin product is available for treatment of patients with symptoms consistent with botulism. However, due to the risks inherent in equine products, treatment is not provided as a result of exposure unless botulism symptoms are present.

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.

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

Clostridium tetani and Tetanus toxin

Clostridium tetani is an anaerobic endospore-forming gram-positive rod found in the soil and 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, high molecular weight protein toxin, consisting of a heavy chain (100kD) subunit that binds the toxin to receptors on neuronal cells and a light chain (50kD) 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 1940's.[62]

Occupational Infections

Although the risk of infection to laboratory personnel is low, there have been five incidents of laboratory personnel exposure recorded.[4]

Natural Modes of Infection

Contamination of wounds by soil is the usual mechanism of transmission for tetanus. Of the 130 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.[63] When introduced into a suitable anaerobic or microaerophilic environment, *C. tetani* spores germinate and produce tetanospasmin. The incubation period ranges from 3 to 21 days. The observed symptoms are primarily associated with the presence of the toxin. Wound cultures are not generally useful for diagnosing tetanus.[64]

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 toxin. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies.

Special Issues

Vaccines The vaccination status of workers should be considered in a risk assessment for workers with this organism and/or toxin. While the risk of laboratory-associated tetanus is low, the administration of an adult diphtheria-tetanus toxoid at 10-year intervals further reduces the risk to laboratory and animal care personnel of toxin exposures and wound contamination, and is therefore highly recommended.[62] The reader is advised to consult the current recommendations of the ACIP. [65]

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

Corynebacterium diphtheriae

Corynebacterium diphtheriae is a pleomorphic gram-positive rod that is isolated from the nasopharynx and skin of humans. The organism is easily grown in the laboratory on media containing 5% sheep blood. *C. diphtheriae* produces a potent exotoxin and is the causative agent of diphtheria, one of the most widespread bacterial diseases in the pre-vaccine era.

Occupational Infections

Laboratory-associated infections with *C. diphtheriae* have been documented, but laboratory animal-associated infections have not been reported.[4,66] 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, larynx, wounds, in blood, and on the skin. Travel to endemic areas or close contact with persons who have returned recently from such areas, increases risk.[67] Transmission usually occurs via direct contact with patients or carriers, and more rarely, with articles 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. An effective vaccine has been developed 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 CIP.[65] While the risk of laboratory-associated diphtheriai is low, the administration of an adult diphtheria-tentanus toxoid at 10-year intervals may further reduce the risk of illness to laboratory and animal care personnel.[62]

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

Francisella tularensis

Francisella tularensis is a small gram-negative coccobacillus that is carried in numerous animal species, especially rabbits, and 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. novicida*, based on virulence testing, 16S sequence, biochemical reactions and epidemiologic features. Type A and Type B strains are highly infectious, requiring only 10-50 organisms to cause disease. Subspecies *F. novicida* is infrequently identified as the cause of human disease. 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 1-4 days with most cases exhibiting symptoms in 3-5 days.[68]

Occupational Infections

Tularemia has been a commonly reported laboratory-associated bacterial infection.[4] Most cases have occurred at facilities involved in tularemia research; however, cases have been reported in diagnostic laboratories as well. Occasional cases were linked to work with naturally or experimentally infected animals or their ectoparasites.

Natural Modes of Infection

Tick bites, 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, cerebrospinal fluid (CSF), blood, urine, 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 has resulted in infection. Infection has been more commonly associated with cultures than with clinical materials and infected animals.[69]

BSL-2 practices, containment equipment, and facilities are recommended for activities involving clinical materials of human or animal origin suspected or known to contain *F. tularensis*. Laboratory personnel should be informed of the possibility of tularemia as a differential diagnosis when samples are submitted for diagnostic tests. 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. Preparatory work on cultures or contaminated materials for automated identification systems should be performed at BSL-3. Characterized strains of reduced virulence such as *F. tularensis* Type B (strain LVS) and *F. tularensis* subsp *novicida* (strain U112) can be manipulated in BSL-2. Manipulation of reduced virulence strains at high concentrations should be conducted using BSL-3 practices.

Special Issues

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 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.

Helicobacter species

Helicobacters are spiral or curved gram-negative rods isolated from gastrointestinal and hepatobiliary tracts of mammals and birds. There are currently 20 recognized species, including at least nine isolated from humans. Since its discovery in 1982, *Helicobacter pylori* has received increasing attention as an agent of gastritis.[70] 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.[71,72] *H. cinaedi* has been isolated from dogs, cats and Syrian hamsters.

Occupational Infections

Both experimental and accidental LAI with *H. pylori* have been reported.[73,74] 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. 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.[75] The enterohepatic helicobacters (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.[72] Protocols involving homogenization or vortexing of gastric specimens have been described for the isolation of *H. pylori*.[76] Containment of potential aerosols or droplets should be incorporated in these procedures.

BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures known to contain or potentially contain the agents. 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 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.

Legionella pneumophila and other Legionella-like Agents

Legionella 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.[77,78] They are able to colonize hot-water tanks at a temperature range from 40 to 50°C. There are currently 48 known *Legionella* species, 20 of which have been associated with human disease. *L. pneumophila* is the species most frequently encountered in human infections.[79-81]

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.[82] Experimental infections have been produced in guinea pigs, mice, rats, embryonated chicken eggs, and human or animal cell lines.[83] A fatal case of pneumonia due to *L. pneumophila* was diagnosed in a calf, but only 1.7% (2/112) of the other cattle in the herd had serological evidence of exposure to *Legionella*.[84] The disease was linked to exposure to a hot water system colonized with *Legionella*. Animal-to-animal transmission has not been demonstrated.

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.[85] Direct person-to-person transmission does not occur. 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.[86] 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 (sputum, pleural fluid, bronchoscopy specimens, lung tissue), and in extrapulmonary sites. A potential hazard may exist for generation of aerosols containing high concentrations of the agent.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of potentially infectious materials, including minimizing the potential for dissemination of the organism from cultures of organisms known to cause disease. 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. For activities likely to produce extensive aerosols and when large quantities of the pathogenic organisms are manipulated, BSL-2 with BSL-3 practices is recommended.

Special Issues

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

Leptospira

The genus *Leptospira* is composed of spiral-shaped bacteria with hooked ends. Leptospires are ubiquitous in nature, either free-living in fresh water 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 12 species based on genetic analysis. These organisms also have been characterized serologically, with more than 200 pathogenic and 60 saprophytic serovars identified as of 2003.[87] 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. Approximately, 70 LAI and 10 deaths have been reported.[4,26] Direct and indirect contact with fluids and tissues of experimentally or naturally infected mammals during handling, care, or necropsy are potential sources of infection.[88-90] 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. Rarely, infection may be transmitted by bites of infected animals.[88]

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 include abrasions, cuts in the skin or via the conjunctiva. Higher rates of infection observed in agricultural workers and other occupations associated with animal contact.

Laboratory Safety and Containment Recommendations

The organism may be present in urine, blood, and tissues of infected animals and humans. Ingestion, accidental 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 not known.

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. The housing and manipulation of infected animals should be performed at ABSL-2. Gloves should be worn to handle and necropsy infected animals and to handle infectious materials and cultures in the laboratory.

Special Issues

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

Listeria monocytogenes

Listeria monocytogenes is a gram-positive, non-spore-forming, aerobic bacillus; that is weakly beta-hemolytic on sheep blood agar and catalase-positive.[91] 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.[91,92] This organism is the causative agent of listeriosis, a food-borne 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.[93] Asymptomatic carriage has been reported in laboratorians.[94]

Natural Modes of Infection

Most human cases of listeriosis result from eating contaminated foods, notably soft cheeses, ready-to-eat meat products (hot dogs, luncheon meats), paté and smoked fish/seafood.[95] 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, *Listeria monocytogenes* infections occur most often in the third trimester and may precipitate labor. Transplacental transmission of *L. monocytogenes* poses a grave risk to the fetus.[92]

Laboratory Safety and Containment Recommendations

Listeria monocytogenes may be found in feces, CSF, and blood, as well as numerous food and environmental samples.[91,92,96,97] 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 the agent. Gloves and eye protection should be worn while handling infected or potentially infected materials. ABSL-2 practices, containment equipment and facilities are recommended for activities involving experimentally or naturally infected animals. Due to potential risks to the fetus, pregnant women should be advised of the risk of exposure to *L. monocytogenes*.

Special Issues

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

Mycobacterium leprae

Mycobacterium leprae is the causative agent of leprosy (Hansen disease). The organism has not been cultivated in laboratory medium but can be maintained in a metabolically active state for some period. Organisms are recovered from infected tissue and can be propagated in laboratory animals, specifically armadillos and the footpads of mice. The infectious dose in humans is unknown. Although naturally occurring leprosy or leprosy-like diseases have been reported in armadillos[98] and in NHP,[99,100] humans are the only known important reservoir of this disease.

Occupational Infections

There are no cases reported as a result of working in a laboratory with biopsy or other clinical materials of human or animal origin. However, inadvertent human-to-human transmissions following an accidental needle stick by a surgeon and after use of a presumably contaminated tattoo needle were reported prior to 1950.[101,102]

Natural Modes of Infection

Leprosy is transmitted from person-to-person following prolonged exposure, presumably via contact with secretions from infected individuals.

Laboratory Safety and Containment Recommendations

The infectious agent 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 accidental parenteral

inoculation are the primary laboratory hazards associated with handling infectious clinical materials. See Appendix B for appropriate tuberculocidal disinfectant.

BSL-2 practices, containment equipment, and facilities are recommended for all activities with known or potentially infectious materials from humans and animals. Extraordinary care should be taken 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 NHP, because coughing with dissemination of infectious droplets does not occur in these species.

Special Issues

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

Mycobacterium tuberculosis complex

The *Mycobacterium tuberculosis* complex includes *M. tuberculosis, M. bovis*, *M. africanum,* and *M. microti* that cause tuberculosis in humans, and more recently recognized *M. caprae* and *M. pinnipedii* that have been isolated from animals. *M. tuberculosis* grows slowly, requiring three weeks for formation of colonies on solid media. The organism has a thick, lipid-rich cell wall that renders bacilli resistant to harsh treatments including alkali and detergents and allows them to stain acid-fast.

Occupational Infections

M. tuberculosis and *M. bovis* infections are a proven hazard to laboratory personnel as well as others who may be exposed to infectious aerosols in the laboratory, autopsy rooms, and other healthcare facilities.[4,26,103-105] The incidence of tuberculosis in laboratory personnel working with *M. tuberculosis* has been reported to be three times higher than that of those not working with the agent.[106] Naturally or experimentally infected NHP are a proven source of human infection.[107] Experimentally infected guinea pigs or 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. Persons infected with *M. tuberculosis* can develop active disease within months of infection or can remain latently infected and develop

disease later in life. The primary focus of infection is the lungs, but most other organs can be involved. HIV infection is a serious risk factor for development of active disease. Infectious aerosols produced by coughing spread tuberculosis. *M. bovis* is primarily found in animals but also can produce tuberculosis in humans. It is spread to humans, primarily children, by consumption of non-pasteurized milk and milk products, by handling of infected carcasses, and by inhalation. Human-to-human transmission via aerosols also 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 hazard encountered. Tubercle bacilli may survive in heat-fixed smears[108] and may be aerosolized in the preparation of frozen sections and during manipulation of liquid cultures. Because of the low infective dose of *M. tuberculosis* (i.e., ID_{50} <10 bacilli), sputa and other clinical specimens from suspected or known cases of tuberculosis must be considered potentially infectious and handled with appropriate precautions. Accidental needle-sticks are also a recognized hazard.

BSL-2 practices and procedures, containment equipment, and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a BSC. Use of a slide-warming tray, rather than a flame, is recommended for fixation of slides. Liquifaction and concentration of sputa for acid-fast staining may be conducted safely on the open bench by first treating the specimen in a BSC with an equal volume of 5% sodium hypochlorite solution (undiluted household bleach) and waiting 15 minutes before processing.[109,110]

BSL-3 practices, containment equipment, and facilities are required for laboratory activities in the propagation and manipulation of cultures of any of the subspecies of the *M. tuberculosis* complex and for animal studies using experimentally or naturally infected NHP. Animal studies using guinea pigs or mice can be conducted at ABSL-2.[111] BSL-3 practices should include the use of respiratory protection and the implementation of specific procedures and use of specialized equipment to prevent and contain aerosols. Disinfectants proven to be tuberculocidal should be used. See Appendix B for additional information.

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 must be exercised to verify the identity of the strain and to ensure that cultures are not contaminated with virulent *M. tuberculosis* or other *M. bovis* strains. Selection of an appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria. See Appendix B for additional information.

Special Issues

Surveillance Annual or semi-annual skin testing with purified protein derivative (PPD) of previously skin-test-negative personnel can be used as a surveillance procedure.

Vaccines The attenuated live BCG, is available and used in other countries but is not used in the United States for immunization.

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

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

More than 100 species of mycobacteria are recognized. These include both slowly growing and rapidly growing species. 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 or mycobacteria other than tuberculosis. Many of the species are common environmental organisms, and approximately 25 of them are associated with infections in humans. A number of additional species are associated with infections in immunocompromised persons, especially HIV-infected individuals. All of these species are considered opportunistic pathogens in humans and none are considered communicable. Mycobacteria are frequently isolated from clinical samples but may not be associated with disease. 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* and *M. scrofulaceum;*
3. disseminated infections in immunocompromised individuals caused by *M. avium*;
4. skin ulcers and soft tissue wound infections including Buruli ulcer caused by *M. ulcerans,* swimming pool granuloma caused by *M. marinum* associated with exposure to organisms in fresh and salt water and fish tanks, and tissue infections resulting from trauma, surgical procedures, or injection of contaminated materials caused by *M. fortuitum, M. chelonei*, and *M. abscesens*.

Occupational Infections

Laboratory-acquired infections with *Mycobacterium* spp. other than *M. tuberculosis* complex have not been reported.

Natural Modes of Infection

Person-to-person transmission has not been demonstrated. Presumably, pulmonary infections are 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. They are also common in potable water supplies, perhaps as the result of the formation of biofilms. The source of *M. avium* infections in immunocompromised persons has not been established.

Laboratory Safety and Containment Recommendations

Various species of mycobacteria may be present in sputa, exudates from lesions, tissues, and in environmental samples. Direct contact of skin or mucous membranes with infectious materials, ingestion, and accidental 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 *Mycobacteria* spp. other than *M. tuberculosis* complex. Clinical specimens may also contain *M. tuberculosis* and care must be exercised to ensure the correct identification of cultures. Special caution should be exercised in handling *M. ulcerans* 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 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.

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.[112]

Occupational Infections

Laboratory-associated gonococcal infections have been reported in the United States and elsewhere.[113-116] 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 2004 rate of reported infections for this disease in the United States was 112 per 100,000 population.[117] 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.[112]

Laboratory Safety and Containment Recommendations

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

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of clinical materials or cultures. Gloves should be worn when handling infected laboratory animals and when there is the likelihood of direct skin contact with infectious materials. 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. Animal studies may be performed at ABSL-2.

Special Issues

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

Neisseria meningitidis

Neisseria meningitidis is a gram-negative coccus responsible for serious acute meningitis and septicemia in humans. Virulence is associated with the expression of a polysaccharide capsule. Thirteen different capsular serotypes have been identified, with types A, B, C, Y, and W135 associated with the highest incidence

of disease. The handling of invasive *N. meningitidis* isolates from blood or CSF represents an increased risk to microbiologists.[118,119]

Occupational Infections

Recent studies of LAI and exposures have indicated that manipulating suspensions of *N. meningitidis* outside a BSC is associated with a high risk for contracting meningococcal disease.[119] Investigations of potential laboratory-acquired cases of meningococcal diseases in the United States showed a many-fold higher attack rate for microbiologists compared to that of the United States general population age 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 had manipulated sterile site isolates on an open laboratory bench.[120] While isolates obtained from respiratory sources are generally less pathogenic and consequently represent lower risk for microbiologists, rigorous protection from droplets or aerosols is mandated when microbiological procedures are performed on all *N. meningitidis* isolates, especially on those from sterile sites.

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 is 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, and saliva. Parenteral inoculation, droplet exposure of mucous membranes, infectious aerosol and ingestion are the primary hazards to laboratory personnel. Based on the mechanism of natural infection and the risk associated with 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.

Specimens for *N. meningitidis* analysis and cultures of *N. meningitidis* not associated with invasive disease may be handled in BSL-2 facilities with rigorous application of BSL-2 standard practices, special practices, and safety equipment. All sterile-site isolates of *N. meningitidis* should be manipulated within a BSC. Isolates of unknown source should be treated as sterile-site isolates.

If a BSC is unavailable, manipulation of these isolates should be minimized, primarily focused on serogroup identification using phenolized saline solution while wearing a laboratory coat, gloves, and safety glasses or full-face splash shield. 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. Animal studies should be performed under ABSL-2 conditions.

Special Issues

Vaccines The quadrivalent meningococcal polysaccharide vaccine, which includes serogroups A, C, Y, and W-135, will decrease but not eliminate the risk of infection, because it is less than 100% effective and does not provide protection against serogroup B, which caused one-half of the laboratory-acquired cases in the United States in 2000.[118,120] Laboratorians who are exposed routinely to potential aerosols of *N. meningitidis* should consider vaccination.[118,121,122]

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

Salmonella serotypes, other than 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 materials. Recent taxonomic studies have organized this genus into two species, *S. enterica* and *S. bongori*, containing more than 2500 antigenically distinct subtypes or serotypes.[123] *S. enterica* contains the vast majority of serotypes associated with human disease. *S. enterica* serotypes Typhimurium and Enteritidis (commonly designated *S. Typhimurium* and *S. Enteritidis*) are the serotypes most frequently encountered in the United States. This summary statement covers all pathogenic serotypes except *S. Typhi*.

Occupational Infections

Salmonellosis is a documented hazard to laboratory personnel.[4,26,124-125] 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 laboratory-acquired infections indicate a presentation of symptoms (fever, severe diarrhea, abdominal cramping) similar to those of naturally-acquired infections, although one case also developed erythema nodosum and reactive arthritis.[126,127]

Natural Modes of Infection

Salmonellosis is a food borne disease of worldwide distribution. An estimated 5 million cases of salmonellosis occur annually in the United States. A wide range of domestic and feral animals (poultry, swine, rodents, cattle, iguanas, turtles,

chicks, dogs, cats) may serve as reservoirs for this disease, as well as humans.[128] The most common mode of transmission is by ingestion of food from contaminated animals or contaminated during processing. The disease usually presents as an acute enterocolitis, with an incubation period ranging from 6 to 72 hours.

Laboratory Safety and Containment Recommendations

The agent may be present in feces, blood, urine, and in food, feed, and environmental materials. Ingestion or parenteral inoculation are the primary laboratory hazards. The importance of aerosol exposure is not known. Naturally or experimentally infected animals are a potential source of infection for laboratory and animal care personnel, and for other animals

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as a BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 facilities and practices are recommended for activities with experimentally infected animals.

Special Issues

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

Salmonella Typhi

Recent taxonomic studies have organized the genus *Salmonella* into two species, *S. enterica* and *S. bongori*, containing more than 2500 antigenically distinct subtypes or serotypes.[123] *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. *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.

Occupational Infections

Typhoid fever is a demonstrated hazard to laboratory personnel.[4,129,130] 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.[131] Laboratory-acquired *S. Typhi* infections usually present with symptoms of septicemia, headache, abdominal pain, and high fever.[129]

Natural Modes of Infection

Typhoid fever is a serious, potentially lethal bloodstream infection of worldwide distribution. Humans are the sole reservoir and asymptomatic carriers may occur. The infectious dose is low (<103 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.[123]

Laboratory Safety and Containment Recommendations

The agent may be present in feces, blood, gallbladder (bile), and urine. Humans are the only known reservoir of infection. Ingestion and parenteral inoculation of the organism represent the primary laboratory hazards. The importance of aerosol exposure is not known.

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. This includes conducting procedures with aerosol or high splash potential in primary containment devices such as BSCs or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. BSL-3 practices and equipment are recommended for activities likely to produce significant aerosols or for activities involving production quantities of organisms. ABSL-2 facilities, practices and equipment are recommended for activities with experimentally infected animals. ABSL-3 conditions may be considered for protocols involving aerosols.

Special Issues

Vaccines Vaccines for *S. Typhi* are available and should be considered for personnel regularly working with potentially infectious materials. The reader is advised to consult the current recommendations of the Advisory Committee on

Immunization Practices (ACIP) published in the CDC Morbidity and Mortality Weekly Report for recommendations for vaccination against *S. Typhi*.[132]

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

Shiga toxin (Verocytotoxin)-producing Escherichia coli

Escherichi coli is one of five 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 intensively studied prokaryotes. An extensive serotyping system has been developed for *E. coli* based 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.

The diarrheagenic *E. coli* strains have been characterized into at least four basic pathogenicity groups: Shiga toxin (Verocytotoxin)-producing *E. coli* (a subset of which are referred to as enterohemorrhagic *E. coli*), enterotoxigenic *E. coli*, enteropathogenic *E. coli*, and enteroinvasive *E. coli*.[123] In addition to clinical significance, *E. coli* strains are commonly-used hosts for cloning experiments and other genetic manipulations in the laboratory. This summary statement provides recommendations for safe manipulation of Shiga toxin-producing *E. coli* strains. Procedures for safely handling laboratory derivatives of *E. coli* or other pathotypes of *E. coli* should be based upon a thorough risk assessment.

Occupational Infections

Shiga toxin-producing *E. coli* strains, including strains of serotype O157:H7, are a demonstrated hazard to laboratory personnel.[133-138] The infectious dose is estimated to be low—similar to that reported for *Shigella* spp., 10-100 organisms.[136] Domestic farm animals (particularly bovines) are significant reservoirs of the organisms; however, experimentally infected small animals are also sources of infection in the laboratory.[139] Verocytotoxin-producing *Escherichia coli* have also been in wild birds and rodents in close proximity to farms.[140]

Natural Modes of Infection

Cattle represent the most common natural reservoir of Shiga-toxin producing *E. coli.* 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, day care centers, and custodial institutions. Water-borne transmission has been reported from outbreaks

associated with swimming in a crowded lake and drinking unchlorinated municipal water.[139] In a small proportion 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 be found in blood or urine specimens from infected humans or animals. Accidental ingestion is the primary laboratory hazard. The importance of aerosol exposure is not known.

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Procedures with aerosol or high splash potential should be conducted with primary containment equipment or in devices such as a BSC or safety centrifuge cups. Personal protective equipment, such as splash shields, face protection, gowns, and gloves should be used in accordance with a risk assessment. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 practices and facilities are recommended for activities with experimentally or naturally infected animals.

Special Issues

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

Shigella

The genus *Shigella* is composed of nonmotile gram-negative bacteria in the family Enterobacteriaceae. There are four subgroups that have been historically treated as separate species, even though more recent genetic analysis indicates that they are members of the same species. These include 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.[123]

Occupational Infections

Shigellosis is one of the most frequently reported laboratory-acquired infections in the United States.[131,141] A survey of 397 laboratories in the United Kingdom revealed that in 1994-1995, four of nine reported laboratory-acquired infections were caused by *Shigella*.[142] Experimentally infected guinea pigs, other rodents, and NHP are proven sources of laboratory-acquired infection.[143,144]

Natural Modes of Infection

Humans and other large primates are the only natural reservoirs of *Shigella* bacteria. Most transmission is by fecal-oral route; infection also is caused by ingestion of contaminated food or water.[123] Infection with *Shigella dysenteriae* type 1 causes more severe, prolonged, and frequently fatal illness than does infection with other *Shigella*. Complications of shigellosis include hemolytic uremic syndrome, which is associated with *S. dysenteriae* 1 infection, and Reiter chronic arthritis syndrome, which is associated with *S. flexneri* infection.

Laboratory Safety and Containment Recommendations

The agent may be present in feces and, rarely, in the blood of infected humans or animals. Accidental ingestion and parenteral inoculation of the agent are the primary laboratory hazards. The 50% infectious dose (oral) of *Shigella* for humans is only a few hundred organisms.[143] The importance of aerosol exposure is not known.

Strict compliance with BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Procedures with aerosol or high splash potential should be conducted with primary containment equipment such as a BSC or safety centrifuge cups. Personal protective equipment should be used in accordance with a risk assessment, including splash shields, face protection, gowns, and gloves. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 facilities and practices are recommended for activities with experimentally or naturally infected animals.

Special Issues

Vaccines Vaccines are currently not available for use in humans.

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

USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

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*.[145] *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* spp. *pallidum* (associated with venereal syphilis), *T. pallidum* spp. *endemicum* (associated with endemic syphilis), and *T. pallidum* spp. *pertenue* (associated with Yaws). These organisms are obligate human pathogens.

Occupational Infections

T. pallidum is a documented hazard to laboratory personnel. Pike lists 20 cases of LAI.[4] Syphilis has been transmitted to personnel working with a concentrated suspension of *T. pallidum* obtained from an experimental rabbit orchitis.[146] *T. pallidum* is present in the circulation during primary and secondary syphilis. The ID_{50} of *T. pallidum* needed to infect rabbits by subcutaneous injection has been reported to be as low as 23 organisms.[147] The concentration of *T. pallidum* in patients' blood during early syphilis, however, has not been determined. No cases of laboratory animal-associated infections are reported; however, rabbit-adapted *T. pallidum* (Nichols strain and possibly others) retains virulence for humans.

Natural Modes of Infection

Humans are the only known natural reservoir of *T. pallidum* and transmission occurs via direct sexual contact (venereal syphilis), direct skin contact (Yaws), or direct mucous contact (endemic syphilis). Venereal syphilis is a sexually transmitted disease that occurs in many areas of the world, 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.[145]

Laboratory Safety and Containment Recommendations

The agent may be present in materials collected from cutaneous and mucosal lesions and in blood. Accidental parenteral inoculation, contact with 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 samples from humans or infected rabbits. Gloves should be worn when there is a likelihood

of direct skin contact with infective materials. Periodic serological monitoring should be considered in personnel regularly working with these materials. 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 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.

Vibrio enteritis species (V. cholerae, V. parahaemolyticus)

Vibrio species are straight or curved motile gram-negative rods. Growth of *Vibrio* species is stimulated by sodium and the natural habitats of these organisms are primarily aquatic environments. Although 12 different *Vibrio* species have been isolated from clinical specimens, *V. cholerae* and *V. parahaemolyticus* are the best-documented causes of human disease.[148] Vibrios may cause either diarrhea or extraintestinal infections.

Occupational Infections

Rare cases of bacterial enteritis due to LAI with either *V. cholerae* or *V. parahaemolyticus* have been reported from around the world.[4] Naturally and experimentally infected animals[149] and shellfish[150,151] are potential sources for such illnesses.

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 10^6-10^{11} colony forming units,[152] while that of *V. parahaemolyticus* ranges from 10^5-10^7 cells.[153] The importance of aerosol exposure is unknown although it has been implicated in at least one instance.[149] 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.[154]

Laboratory Safety and Containment Recommendations

Pathogenic vibrios can be present in human fecal samples, or in the meats and the exterior surfaces of marine invertebrates such as shellfish. Other clinical specimens from which vibrios may be isolated include blood, arm or leg wounds,

eye, ear, and gallbladder.[148] Accidental oral ingestion of *V. cholerae* or *V. parahaemolyticus* principally results from hands contaminated from the use of syringes or the handling of naturally contaminated marine samples without gloves.

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 The reader is advised to consult the current recommendations of the ACIP published in the MMWR for vaccination recommendations against *V. cholera*. There are currently no human vaccines against *V. parahaemolyticus*.

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

Yersinia pestis

Yersinia pestis, the causative agent of plague, is a gram-negative, microaerophilic coccobacillus frequently characterized by a “safety pin” appearance on stained preparations from specimens. It is nonmotile and nonsporulating. There are three biotypes of *Y. pestis,* differentiated by their ability to ferment glycerol and reduce nitrate. All three biotypes are virulent. The incubation period for bubonic plague ranges from two to six days while the incubation period for pneumonic plague is one to six days. Pneumonic plague is transmissible person-to-person;[155] whereas bubonic plague is not. Laboratory animal studies have shown the lethal and infectious doses of *Y. pestis* to be quite low (less than 100 colony forming units).[156]

Occupational Infections

Y. pestis is a documented laboratory hazard. Prior to 1950, at least 10 laboratory-acquired cases were reported in the United States, four of which were fatal.[4,157] Veterinary staff and pet owners have become infected when handling domestic cats with oropharyngeal or pneumonic plague.

Natural Modes of Infection

Infective fleabites are the most common mode of transmission, but direct human contact with infected tissues or body fluids of animals and humans also may serve as sources of infection.

Primary pneumonic plague arises from the inhalation of infectious respiratory droplets or other airborne materials from infected animals or humans. This form of plague has a high case fatality rate if not treated and poses the risk of person-to-person transmission.

Laboratory Safety and Containment Recommendations

The agent has been isolated, in order of frequency of recovery, from bubo aspirate, blood, liver, spleen, sputum, lung, bone marrow, CSF, and infrequently from feces and urine, depending on the clinical form and stage of the disease. 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 and field personnel should be counseled on methods to avoid fleabites and accidental autoinoculation when handling potentially infected live or dead animals.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the handling of potentially infectious clinical materials and cultures. In addition, because the infectious dose is so small, all work, including necropsies of potentially infected animals should be performed in a BSC. Special care should be taken to avoid generating aerosols or airborne droplets while handling infectious materials or when performing necropsies on naturally or experimentally infected animals. Gloves should be worn when handling potentially infectious materials including field or laboratory infected animals. BSL-3 is recommended for activities with high potential for droplet or aerosol production, and for activities involving large-scale production or high concentrations of infectious materials. Resistance of *Y. pestis* strains to antibiotics used in the treatment of plague should be considered in a thorough risk assessment and may require additional containment for personal protective equipment. For animal studies, a risk assessment that takes into account the animal species, infective strain, and proposed procedures should be performed in order to determine if ABSL-2 or ABSL-3 practices, containment equipment, and facilities should be employed. BSL-3 facilities and arthropod containment level 3 practices are recommended for all laboratory work involving infected arthropods.[157] See Appendix G for additional information on arthropod containment guidelines.

Special Issues

Select Agent *Yersinia pestis* is an HHS select agent requiring registration with CDC for the possession, use, storage and transfer. See Appendix F for further information.

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

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Section VIII-B: Fungal Agents

Blastomyces dermatitidis

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; these 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. The sexual stage is an Ascomycete with infectious ascospores.

Occupational Infections

Three groups are at greatest risk of laboratory-acquired infection: microbiologists, veterinarians and pathologists.[1] Laboratory-associated local infections have been reported following accidental parenteral inoculation with infected tissues or cultures containing yeast forms of *B. dermatitidis*.[2-8] Pulmonary infections have occurred following the presumed inhalation of conidia from mold-form cultures; two persons developed pneumonia and one had an osteolytic lesion from which *B. dermatitidis* was cultured.[9,10] Presumably, pulmonary infections are associated only with sporulating mold forms.

Natural Modes of Infection

The fungus has been reported from multiple geographically separated countries, but 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.[11]

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-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials, animal tissues, yeast-form cultures, and infected animals. BSL-3 practices, containment equipment, and facilities are required for handling sporulating mold-form cultures already identified as *B. dermatitidis* and soil or other environmental samples known or likely to contain infectious conidia.

Special Issues

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

Coccidioides immitis and Coccidioides posadasii

Coccidioides spp. is endemic to lower sonoran deserts of the western hemisphere including northern Mexico, southern Arizona, central and southern California, and west Texas. The original species (*C. immitis*) has been divided into *C. immitis* and *C. posadasii*.[12] 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 that 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.[13-15] Occupational exposure has also been associated in endemic regions with archeology[16] and high dust exposure.[17] Attack rates for laboratory and occupational exposure are higher than for ambient exposure when large numbers of spores are inhaled. Smith reported that 28 of 31 (90%) laboratory-associated infections in his institution resulted in clinical disease, whereas more than half of infections acquired in nature were asymptomatic.[18] Risk of respiratory infection from exposure to infected tissue or aerosols of infected secretions is very low. Accidental percutaneous inoculation has typically resulted in local granuloma formation.[19]

Natural Modes of Infection

Single spores can produce ambient infections by the respiratory route. Peak exposures occur during arid seasons. *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.

The majority of ambient infections is subclinical and results in life-long protection from subsequent exposures. The incubation period is one to three weeks and manifests as a 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 is 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 (AIDS, organ transplant recipient, lymphoma).

Laboratory Safety and Containment Recommendations

Because of their size, the 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 in mold cultures and soil or other samples from natural sites. Inhalation of arthroconidia from environmental samples or cultures of the mold form is a serious laboratory hazard. Personnel should be aware that infected animal or human clinical specimens or tissues stored or shipped in such a manner as to promote germination of arthroconidia pose a theoretical laboratory hazard.

BSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, and processing animal tissues. ABSL-2 practices, containment equipment, and facilities are recommended for experimental animal studies when the route of challenge is parenteral.

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 to contain infectious arthroconidia. Experimental animal studies should be done at BSL-3 when challenge is via the intranasal or pulmonary route.

Special Issues

Select Agent Some *Coccidioides* spp. are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

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

Cryptococcus Neoformans

Cryptococcus neoformans is a monomorphic fungal pathogen existing in nature, in laboratory cultures at room temperature and *in vivo* as a budding yeast. The sexual stage is grouped with the Basidiomycetes and is characterized by sparse

hyphal formation with basidiospores. Both basidiospores and asexual yeasts are infectious.

Occupational Infections

Accidental inoculation of a heavy inoculum of *C. neoformans* into the hands of laboratory workers has occurred during injection or necropsy of laboratory animals.[20,21] Either a local granuloma or no lesion was reported, suggesting low pathogenicity by this route. Respiratory infections as a consequence of laboratory exposure have not been recorded.

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with pigeon feces. Infections are not transmissible from person-to-person, but require common exposure via the respiratory route to a point source.

Laboratory Safety and Containment Recommendations

Accidental parenteral inoculation of cultures or other infectious materials represents a potential hazard to laboratory personnel, particularly to those who may be immunocompromised. Bites by experimentally infected mice and manipulations of infectious environmental materials (e.g., pigeon feces) may also represent a potential hazard to laboratory personnel. *C. neoformans* has been isolated from bedding of cages housing mice with pulmonary infection indicating the potential for contamination of cages and animal facilities by infected animals.[22] Reports of cutaneous cryptococcal infection following minor skin injuries suggests that localized infection may complicate skin injuries incurred in laboratories that handle *C. neoformans*.[23]

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with known or potentially infectious clinical, environmental, or culture materials and with experimentally infected animals. This agent and any samples that may contain this agent should also be handled in a Class II BSC.

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

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 (conidia); these 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.

Occupational Infections

Laboratory-associated histoplasmosis is a documented hazard in facilities conducting diagnostic or investigative work.[24-27] 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] and from spray from a needle into the eye.[32] Collecting and processing soil samples from endemic areas has caused pulmonary infections in laboratory workers.[33] Conidia are resistant to drying and may remain viable for long periods of time. The small size of the infective conidia (less than 5 microns) is conducive to airborne dispersal and intrapulmonary retention. Work with experimental animals suggests that hyphal fragments are capable of serving as viable inocula.[24]

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with starling 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 particles, usually microconidia.[24] Infections are not transmissible from person-to-person, but require common exposure to a point source.

Laboratory Safety and Containment Recommendations

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

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 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.

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

Special Issues

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

Sporothrix schenckii

Sporothrix schenckii is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia); these are the infectious particles that convert to small budding yeasts in the parasitic phase *in vivo*. 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, *S. schenckii* has caused a substantial number of local skin or eye infections in laboratory personnel.[34] Most occupational cases have been associated with accidents and have involved splashing culture material into the eye,[35,36] scratching,[37] or injecting[38] infected material into the skin or being bitten by an experimentally infected animal.[39,40] Skin infections in the absence of trauma have resulted also from handling cultures[41-43] or necropsy of animals[44] without any apparent trauma.

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with sphagnum moss and gardening, often involving sphagnum moss and traumatic implantation. Infections are not transmissible from person-to-person, but require common exposure to a point source. Rare respiratory and zoonotic infections occur. It is thought that naturally occurring lung disease results from inhalation.

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 from laboratory exposure. It should be noted that serious disseminated infections have been reported in immunocompromised persons.[45]

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for laboratory handling of suspected clinical specimens, soil and vegetation, and experimental animal activities with *S. schenckii.* Gloves should

be worn during manipulation of *S. schenckii* and when handling experimentally infected animals. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

Special Issues

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

Dermatophytes (Epidermophyton, Microsporum, and Trichophyton)

The dermatophytes are biologically related species of the genera, *Epidermophyton, Microsporum,* and *Trichophyton* that exist as monomorphic pathogens in nature, in laboratory cultures at room temperature and *in vivo* as filamentous molds. The sexual stages, when known, are Ascomycetes with infectious ascospores. These fungi are distributed worldwide, with particular species being endemic in particular regions. The species are grouped by natural environment habitat as being primarily associated with humans (anthrophilic), other animals (zoophilic), or soil (geophilic).

Occupational Infections

Although skin, hair, and nail infections by these molds are among the most prevalent of human infections, the processing of clinical material has not been associated with laboratory infections. Infections have been acquired through contacts with naturally or experimentally infected laboratory animals (mice, rabbits, guinea pigs, etc.) and, occasionally, with handling cultures.[26,29,45,46]

Systemic dermatophytosis is a rare condition. Superficial chronic infections occur frequently among immunocompromised individuals as well as elderly and diabetic persons. Susceptible individuals should use extra caution.[47-50]

Natural Modes of Infection

Infections can be transmissible from person-to-person, or acquired from common exposure to a point source. The dermatophytes cause infection (dermatophytosis) by invading the keratinized tissues of living animals and are among the most common infectious agents of humans. This fungal group encompasses members of three genera: *Epidermophyton, Microsporum,* and *Trichophyton*. The severity of infection depends on the infective species or strain, the anatomic site and other host factors. One of the most severe dermatophytoses is favus, a disfiguring disease of the scalp caused by *Trychophyton schoenleinii*.

Laboratory Safety and Containment Recommendations

Dermatophytes pose a moderate potential hazard to individuals with normal immune status. In the clinical laboratory setting, the inappropriate handling of cultures is the most common source of infection for laboratory personnel. The most common laboratory procedure for detection of the infective dermatophyte is the direct microscopic examination of contaminated skin, hair, and nails, followed by its isolation and identification on appropriated culture media. Direct contact with contaminated skin, hair, and nails of humans could be another source of infection.[48,49] In research laboratories, dermatophytosis can be acquired by contact with contaminated soil (source of infection: geophilic species) or animal hosts (source of infection: zoophilic species).

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for handling cultures and soil samples. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

Special Issues

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

Miscellaneous Molds

Several molds have caused serious infection in immunocompetent hosts following presumed inhalation or accidental subcutaneous inoculation from environmental sources. These agents include the dimorphic mold, *Penicillium marneffei*, and the dematiaceous (brown-pigmented) molds, *Bipolaris* species, *Cladophialophora bantiana*, *Exophiala (Wangiella) dermatitidis*, *Exserohilum* species, *Fonsecaea pedrosoi, Ochroconis gallopava (Dactylaria gallopava), Ramichloridium mackenziei (Ramichloridium obovoideum), Rhinocladiella atrovirens*, and *Scedosporium prolificans*.[51]

Occupational Infections

Even though no laboratory-acquired infections appear to have been reported with most of these agents, the gravity of naturally-acquired illness is sufficient to merit special precautions in the laboratory. *Penicillium marneffei* has caused a localized infection in a laboratory worker.[52] It also caused a case of laboratory-acquired disseminated infection following presumed inhalation when an undiagnosed HIV-positive individual visited a laboratory where students were handling cultures on the open bench.[53]

Natural Modes of Infection

The natural mode of infection varies by specific species; most are poorly characterized.

Laboratory Safety and Containment Recommendations

Inhalation of conidia from sporulating mold cultures or accidental injection into the skin during infection of experimental animals are potential risks to laboratory personnel.

BSL-2 practices, containment equipment, and facilities are recommended for propagating and manipulating cultures known to contain these agents. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

Special Issues

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

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Section VIII-C: Parasitic Agents

General Issues

Additional details about occupationally-acquired cases of parasitic infections, as well as recommendations for post exposure management, are provided elsewhere.[1-3] Effective antimicrobial treatment is available for most parasitic infections.[4] Immunocompromised persons should receive individualized counseling (specific to host and parasite factors) from their personal healthcare provider and their employer about the potential risks associated with working with live organisms.

BSL-2 and ABSL-2 practices,[5] containment equipment, and facilities are recommended for activities with infective stages of the parasites discussed in this chapter.

Microsporidia, historically considered parasites, are now recognized by most experts to be fungi; however, microsporidia are maintained in the parasitic agent section is this edition. These organisms are discussed here because a laboratory-acquired case of infection has been reported,[6] and most persons currently still look for microsporidia associated with discussion of parasitic agents.

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

Blood and Tissue Protozoal Parasites

Blood and tissue protozoal parasites that pose greatest occupational risk include *Babesia*, *Leishmania, Plasmodium, Toxoplasma*, and *Trypanosoma*. Other tissue protozoa of potential concern include free-living ameba (*Acanthamoeba*, *Balamuthia mandrillaris*, *Naegleria fowleri*) and some species of microsporidia including *Encephalitozoon cuniculi* that commonly cause extraintestinal infection.

Leishmania spp. cause human leishmaniasis; *Plasmodium* spp. cause human malaria, or some, such as *P. cynomolgi* cause nonhuman primate malaria; *Toxoplasma gondii* causes toxoplasmosis; *Trypanosoma cruzi* causes American trypanosomiasis or Chagas disease; and *Trypanosoma brucei gambiense* and *T. b. rhodesiense* cause African trypanosomiasis or (African) sleeping sickness. With the exception of *Leishmania* and *Toxoplasma*, these agents are classically thought of as bloodborne and have stages that circulate in the blood. Although not always recognized, both *Leishmania* and *Toxoplasma* may have stages that circulate in the blood. Some, such as *Plasmodium* and *Trypanosoma cruzi*, also have tissue stages. *Leishmania* spp. are well recognized to have skin and deep tissue stages and *Toxoplasma gondii* forms tissue cysts, including in the central nervous system.

Occupational Infections

Laboratory-acquired infections with *Leishmania* spp.*, Plasmodium* spp.*, Toxoplasma gondii*, and *Trypanosoma* spp. have been reported; the majority of these involved needle-stick or other cutaneous exposure to infectious stages of the organisms through abraded skin, including microabrasions.[1,2]

Laboratory-acquired infections may be asymptomatic. If clinically manifest, they may exhibit features similar to those seen in naturally acquired infections, although bypassing natural modes of infection could result in atypical signs and symptoms. Cutaneous leishmaniasis could manifest as various types of skin lesions (e.g., nodules, ulcers, plaques), while visceral leishmaniasis may result in fever, hepatosplenomegaly, and pancytopenia. However, only one of the laboratorians known to have become infected with *L. (L.) donovani*, an organism typically associated with visceral leishmaniasis, developed clinical manifestations of visceral involvement (e.g., fever, splenomegaly, leukopenia).[1] The other laboratorians developed skin lesions. Laboratory-acquired malaria infections may result in fever and chills, fatigue, and hemolytic anemia. Laboratorians can become infected with *T. gondii* through accidental ingestion of sporulated oocysts, but also may become infected through skin or mucous membrane contact with either tachyzoites or bradyzoites in human or animal tissue or culture. Symptoms in laboratory-acquired *T. gondii* infections may be restricted to flu-like conditions with enlarged lymph nodes, although rash may be present. *Trypanosoma cruzi* infection could manifest initially as swelling and redness at the inoculation site, fever, rash, and adenopathy. Myocarditis and electrocardiographic changes may develop. Infection with *T. b. rhodesiense* and *T. b. gambiense* also may cause initial swelling and redness at the inoculation site, followed by fever, rash, adenopathy, headache, fatigue and neurologic signs.

Blood and tissue protozoal infections associated with exposure to laboratory animals are not common. Potential direct sources of infection for laboratory personnel include accidental needle-stick while inoculating or bleeding animals, contact with lesion material from cutaneous leishmaniasis, and contact with blood of experimentally or naturally infected animals. In the case of rodents experimentally inoculated with *Toxoplasma gondii* via the intraperitoneal route, contact with peritoneal fluid could result in exposure to infectious organisms. Mosquito-transmitted malaria infections can occur under laboratory conditions as nearly half of the occupationally acquired malaria infections were reported to be vector borne, and contact with body fluids (including feces) of reduviids (triatomines) experimentally or naturally infected with *T. cruzi* poses a risk to laboratory personnel.

Babesia microti and other *Babesia* spp. can cause human babesiosis or piroplasmosis. Under natural conditions, *Babesia* is transmitted by the bite of an infected tick, or by blood transfusion; in the United States, hard ticks (*Ixodes)* are the principal vectors. Although no laboratory infections with *Babesia* have been

reported, they could easily result from accidental needle-stick or other cutaneous exposure of abraded skin to blood containing parasites. Persons who are asplenic, immunocompromised, or elderly have increased risk for severe illness if infected.

Natural Modes of Infection

Leishmaniasis is endemic in parts of the tropics, subtropics, and southern Europe, while malaria is widely distributed throughout the tropics. However, the prevalence of these diseases varies widely among endemic areas; the diseases can be very focal in nature. The four species of malaria that infect humans have no animal reservoir hosts. Some *Leishmania* spp. may have a number of important mammalian reservoir hosts, including rodents and dogs. Only cats and other felines can serve as definitive hosts for *Toxoplasma gondii*, which is distributed worldwide. Birds and mammals, including sheep, pigs, rodents, cattle, deer, and humans can be infected from ingestion of tissue cysts or fecal oocysts and subsequently develop tissue cysts throughout the body. Chagas disease occurs from Mexico southward throughout most of Central and South America, with the exception of the southern-most tip of South America. It has been characterized in some accounts as a zoonotic infection, yet the role of animals in maintaining human infection is unclear. A variety of domestic and wild animals are found naturally infected with *T. cruzi*, but human infection undoubtedly serves as the major source of infection for other humans. African trypanosomiasis is endemic in sub-Saharan Africa but is extremely focal in its distribution. Generally, *T. b. gambiense* occurs in West and Central Africa while *T. b. rhodesiense* occurs in East and Southeast 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*.

Leishmania, *Plasmodium*, and both American and African trypanosomes are all transmitted in nature by blood-sucking insects. Sandflies in the genera *Phlebotomus* and *Lutzomyia* transmit *Leishmania*; mosquitoes in the genus *Anopheles* transmit *Plasmodium*; reduviid (triatomine) bugs such as *Triatoma*, *Rhodnius*, and *Panstrongylus* transmit *T. cruzi* (in the feces rather than the saliva of the bug), and tsetse flies in the genus *Glossina* transmit African trypanosomes.

Laboratory Safety and Containment Recommendations

Infective stages may be present in blood, CSF, bone marrow, or other biopsy tissue, lesion exudates, and infected arthropods. Depending on the parasite, the primary laboratory hazards are skin penetration through wounds or microabrasions, accidental parenteral inoculation, and transmission by arthropod vectors. Aerosol or droplet exposure of organisms to the mucous membranes of the eyes, nose, or mouth are potential hazards when working with cultures of

Leishmania, *Toxoplasma gondii*, or *T. cruzi*, or with tissue homogenates or blood containing hemoflagellates. Immuno-compromised persons should avoid working with live organisms.

Because of the potential for grave consequences of toxoplasmosis in the developing fetus, women who are or might become pregnant and who are at risk for infection with *T. gondii* should receive counseling from their personal physician and employer regarding appropriate means of mitigating the risk (including alternate work assignments, additional PPE, etc.). Working with infectious oocysts poses the greatest risk of acquiring infection; needle-sticks with material containing tachyzoites or bradyzoites also pose a significant risk. Infection with tachyzoites or bradyzoites through mucous membranes or skin abrasions is also possible. Kittens and cats that might be naturally infected with *Toxoplasma* pose some risk to personnel.[5] Good hygiene and use of personal protection measures would reduce the risk.

One laboratory infection with microsporidia has been reported, associated with conjunctival exposure to spores leading to the development of keratoconjunctivitis. Infection could also result from ingestion of spores in feces, urine, sputum, CSF, or culture. No laboratory-acquired infections have been reported with *Acanthamoeba* spp., *Balamuthia mandrillaris* or *Naegleria fowleri*; however, the possibility of becoming infected by inhalation, by accidental needle-sticks, or through exposure to mucous membranes or microabrasions of the skin should be considered.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the parasites listed.[5] Infected arthropods should be maintained in facilities that reasonably preclude the exposure of personnel or the escape of insects. (See Appendix E.) Personal protection (e.g., lab coat, gloves, face shield), in conjunction with containment in a BSC, is indicated when working with cultures, tissue homogenates, or blood containing organisms.

Special Issues

Treatment Highly effective medical treatment for most protozoal infections exists.[4] An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.

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

Intestinal Protozoal Parasites

Intestinal protozoal parasites that pose greatest occupational risk include *Cryptosporidium*, *Isospora, Entamoeba histolytica*, and *Giardia*. Other intestinal pathogens of concern are some species of microsporidia, specifically *Septata intestinalis* and *Enterocytozoon bieneusi*. *Cryptosporidium parvum, C. hominis,* and *Isospora belli* cause intestinal coccidiosis, most often referred to as cryptosporidiosis and isosporiasis, respectively. *Entamoeba histolytica* can cause both intestinal and extraintestinal infection (e.g., liver abscess) called amebiasis, and *Giardia intestinalis* causes giardiasis.

Occupational Infections

Laboratory-acquired infections with *Cryptosporidium* spp.*, E. histolytica*, *G. intestinalis,* and *I. belli* have been reported.[1-3] The mode of exposure in laboratory-acquired infections in this group of agents mimics the natural infection routes for the most part, and consequently, clinical symptoms are typically very similar to those seen in naturally acquired infections. For C*ryptosporidium*, *E. histolytica*, *G. intestinalis*, and *I. belli*, the common clinical manifestations are symptoms of gastroenteritis (e.g., diarrhea, abdominal pain and cramping, loss of appetite). Infection with *E. histolytica* may result in bloody stools.

Laboratory animal-associated infections with this group of organisms have been reported and provide a direct source of infection for laboratory personnel who are exposed to feces of experimentally or naturally infected animals.[3] Handling *Cryptosporidium* oocysts requires special care, as laboratory-acquired infections have occurred commonly in personnel working with this agent, especially if calves are used as the source of oocysts. Other experimentally infected animals pose potential risks as well. Circumstantial evidence suggests that airborne transmission of oocysts of this small organism (i.e., 4-6 µm diameter) may occur. Rigid adherence to protocol should reduce the occurrence of laboratory-acquired infection in laboratory and animal care personnel.

Natural Modes of Infection

All of these intestinal protozoa have a cosmopolitan distribution, and in some settings, including developed countries, the prevalence of infection can be high. The natural mode of infection for this group of organisms is typically ingestion of an environmentally hardy oocyst (for the coccidia) or cyst (for *E. histolytica* and *G. intestinalis*). The ID_{50}, best established for *Cryptosporidium*, has been shown for some strains to be 5-10 oocysts.[7] This suggests that even a single oocyst might pose a risk for infection in an exposed laboratorian. The infectious dose for other parasites in this group is not as well established, but is probably in the same range. Further, because these protozoa multiply in the host, ingestion of even small inocula can cause infection and illness. The role for animal reservoir hosts is diverse in this group of organisms. In the case of *C. hominis*, principally humans are infected, whereas for *C. parvum*, humans, cattle, and other

mammals can be infected and serve as reservoir hosts for human infection. In the case of *E. histolytica*, humans serve as the only significant source of infection, and there is no convincing evidence that any animal serves as reservoir host for *I. belli*. The extent to which *Giardia* spp. parasitizing animals can infect humans is only now becoming better understood, but most human infection seems to be acquired from human-to-human transmission. The organisms in this group do not require more than one host to complete their life cycle because they infect, develop, and result in shedding of infectious stages all in a single host. Ingestion of contaminated drinking or recreational water has also been a common source of cryptosporidiosis and giardiasis.

Laboratory Safety and Containment Recommendations

Infective stages may be present in the feces or other body fluids and tissues. Depending on the parasite, ingestion is the primary laboratory hazard. Immunocompromised persons should avoid working with live organisms. Laboratorians who work only with killed or inactivated parasite materials, or parasite fractions, are not at significant risk.

Similarly, no accidental laboratory infection with *Sarcocystis* has been reported, although care should be exercised when working with infected meat products to avoid accidental ingestion. It is not known if laboratorians could be accidentally infected through parenteral inoculation of *Sarcocystis*; nevertheless caution should be exercised when working with cultures, homogenates, etc.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with infective stages of the parasites listed.[5] Primary containment (e.g., BSC) or personal protection (e.g., face shield) is especially important when working with *Cryptosporidium.* Oocysts are infectious when shed (i.e., are already sporulated and do not require further development time outside the host), often are present in stool in high numbers, and are environmentally hardy.

Commercially available iodine-containing disinfectants are effective against *E. histolytica* and *G. intestinalis*, when used as directed, as are high concentrations of chlorine (1 cup of full-strength commercial bleach [~5% chlorine] per gallon of water [1:16, vol/vol]).[1,2]

If a laboratory spill contains *Cryptosporidium* oocysts, the following approach is recommended.[2] A conventional laboratory detergent/cleaner should be used to remove contaminating matter from surfaces (e.g., of bench tops and equipment). After organic material has been removed, 3% hydrogen peroxide (i.e., undiluted, commercial hydrogen peroxide, identified on the bottle as 3% or “10 vol” hydrogen peroxide) can be used to disinfect surfaces; dispensing bottles that contain undiluted hydrogen peroxide should be readily available in laboratories in which surfaces could become contaminated.

Affected surfaces should be flooded (i.e., completely covered) with hydrogen peroxide. If a large volume of liquid contaminates surfaces, to avoid diluting the hydrogen peroxide, absorb the bulk of the spill with disposable paper towels. Dispense hydrogen peroxide repeatedly, as needed, to keep affected surfaces covered (i.e., wet/moist) for ~30 minutes. Absorb residual hydrogen peroxide with disposable paper towels and allow surfaces to dry thoroughly (10 to 30 minutes) before use. All paper towel litter and other disposable materials should be autoclaved or similarly disinfected 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, immerse contaminated items for ~1 hour in a water bath preheated to 50º C; thereafter, wash them in a detergent/disinfectant solution.

Special Issues

Treatment Highly effective medical treatment exists for most protozoal infections; treatment with nitazoxanide for *Cryptosporidium* is now available, but efficacy has not been proven.[4]

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

Trematode Parasites

Trematode parasites that pose greatest occupational risk are the *Schistosoma* spp., although others including *Fasciola* are of concern. *Schistosoma mansoni* causes intestinal schistosomiasis or bilharziasis, also known as Manson's blood fluke, in which the adult flukes reside in the venules of the bowel and rectum. *Fasciola hepatica*, the sheep liver fluke, causes fascioliasis, where the adult flukes live in the common and hepatic bile ducts of the human or animal host.

Occupational Infections

Laboratory-acquired infections with *S. mansoni* and *F. hepatica* have been reported, but accidental infections with other *Schistosoma* spp. could also occur.[1,2] By nature of the infection, none have been directly associated with laboratory animals, with the exception of infected mollusk intermediate hosts.

Laboratory-acquired infections with *F. hepatica* may be asymptomatic, but could have clinical manifestations such as right upper quadrant pain, biliary colic, obstructive jaundice, elevated transaminase levels, and other pathology associated with hepatic damage resulting from migration of the fluke through the liver en route to the bile duct. Most laboratory exposures to schistosomes would

result in predictably low worm burdens with minimal disease potential. However, clinical manifestations of infection with *S. mansoni* could include dermatitis, fever, cough, hepatosplenomegaly, and adenopathy.

Natural Modes of Infection

Fasciola hepatica has a cosmopolitan distribution and is most common in sheep-raising areas, although other natural hosts include goats, cattle, hogs, deer, and rodents. Snails in the family Lymnaeidae, primarily species of *Lymnaea*, are intermediate hosts for *F. hepatica*, and release cercariae that encyst on vegetation. Persons become infected with *F. hepatica* by eating raw or poorly cooked vegetation, especially green leafy plants such as watercress, on which metacercariae have encysted.

Schistosoma mansoni is widely distributed in Africa, South America, and the Caribbean; the prevalence of infection has been rapidly changing in some areas. Infection occurs when persons are exposed to free-swimming cercariae in contaminated bodies of water; cercariae can penetrate intact skin. 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 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 membrane 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 reported cases of laboratory-acquired schistosomiasis have been caused by *S. mansoni*, which probably reflects the fact that many more laboratories work with *S. mansoni* than with other *Schistosoma* spp. However, accidental infection with *S. haematobium*, *S. japonicum*, and *S. mekongi* could easily occur in the same manner as described for *S. mansoni*.

Exposure to cercariae of non-human species of schistosomes (e.g., avian species) may cause mild to severe dermatitis (swimmer's itch).

BSL-2 and ABSL-2 practices, containment equipment and facilities are recommended for laboratory work with infective stages of the parasites listed.[5] Gloves should be worn when there may be direct contact with water containing cercariae or vegetation with encysted metacercariae from naturally or experimentally infected snail intermediate hosts. Long-sleeved laboratory coats or other protective garb should be worn when working in the immediate area of

aquaria or other water sources that may contain schistosome cercariae. Water from laboratory aquaria containing snails and cercariae should be decontaminated (e.g., ethanol, hypochlorite, iodine, or heat) before discharged to sanitary sewers.

Special Issues

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

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

Cestode Parasites

Cestode parasites of potential risk for laboratorians include *Echinococcus* spp., *Hymenolepis nana,* and *Taenia solium.* Echinococcosis is an infection 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 produces hymenolepiasis, or intestinal infection with the adult tapeworm. *Taenia solium*, the pork tapeworm, causes both taeniasis (infection of the intestinal tract with the adult worm), and cysticercosis (infection of subcutaneous, intermuscular, and central nervous system with the metacestode stage or cysticercus).

Occupational Infections

No laboratory-acquired infections have been reported with any cestode parasite.

Natural Modes of Infection

The infectious stage of *Echinococcus, Hymenolepis*, and *Taenia* is the oncosphere contained within the egg. *Hymenolepis nana* is a one-host parasite and does not require an intermediate host; it is directly transmissible by ingestion of feces of infected humans or rodents. The life cycles of *Echinococcus* and *Taenia* require two hosts. Canids, including dogs, wolves, foxes, coyotes, and jackals, are the definitive hosts for *E. granulosus*, and various herbivores such as sheep, cattle, deer, and horses are the intermediate hosts. Foxes and coyotes are the principal definitive hosts for *E. multilocularis*, although dogs and cats also can become infected and rodents serve as the intermediate hosts. Bush dogs and pacas serve as the definitive and intermediate hosts, respectively, for *E. vogeli*. Dogs also may be infected. *Echinococcus oligarthrus* uses wild felines,

including cougar, jaguarondi, jaguar, ocelot, and pampas cat, as definitive hosts and various rodents such as agoutis, pacas, spiny rats, and rabbits serve as intermediate hosts. People become infected when eggs shed by the definitive host are accidentally ingested. For *T. solium*, people can serve both as definitive host (harbor the adult tapeworm), and as accidental intermediate host (harbor the larval stages cysticerci). Pigs are the usual intermediate host, becoming infected as they scavenge human feces containing eggs.

Laboratory Safety and Containment Recommendations

Infective eggs of *Echinococcus* spp. may be present in the feces of carnivore definitive hosts.[3] *Echinococcus granulosus* poses the greatest risk because it is the most common and widely distributed species, 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 from these sources is the primary laboratory hazard. Ingestion of cysticerci of *T. solium (Cysticercus cellulosae)* leads to human infection with the adult tapeworm. For those cestodes listed, the ingestion of a single infective egg from the feces of the definitive host could potentially result in serious disease. Ingestion of the eggs of *H. nana* in the feces of definitive hosts (humans or rodents) could result in intestinal infection.

Although no laboratory-acquired infections with either *Echinococcus* spp. or *T. solium* have been reported, the consequences of such infections could be serious. Laboratory-acquired infections with cestodes could result in various clinical manifestations, depending upon the type of cestode. Human infection with *Echinococcus* spp. could range from asymptomatic to severe. The severity and nature of the signs and symptoms depends upon the location of the cysts, their size, and condition (alive versus dead). Clinical manifestations of a liver cyst could include hepatosplenomegaly, right epigastric pain, and nausea, while a lung cyst may cause chest pain, dyspnea, and hemoptysis. For *T. solium*, ingestion of eggs from human feces can result in cysticercosis, with cysts located in subcutaneous and intermuscular tissues, where they may be asymptomatic. Cysts in the central nervous system may cause seizures and other neurologic symptoms. Ingestion of tissue cysts of *T. solium* can lead to development of adult worms in the intestine of humans. Immunocompromised persons working with these cestodes must take special care as the asexual multiplication of the larval stages of these parasites makes them especially dangerous to such persons.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for work with infective stages of these parasites.[5] Special attention should be given to personal hygiene (e.g., hand washing) and laboratory practices that would reduce the risk of accidental ingestion of infective eggs. Gloves are recommended when there may be direct contact with feces or with surfaces contaminated with fresh feces of carnivores infected with *Echinococcus* spp., humans infected with *T. solium*, or humans or rodents infected with *H. nana*.

Special Issues

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

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

Nematode Parasites

Nematode parasites that pose greatest occupational risk include the ascarids, especially *Ascaris* and *Baylisascaris*; hookworms, both human and animal; *Strongyloides*, both human and animal; *Enterobius*; and the human filariae, primarily *Wuchereria* and *Brugia*. *Ancylostoma braziliense* and *A. caninum* cause hookworm infection in cats and dogs, respectively. *Ascaris lumbricoides* causes ascariasis and is known as the large intestinal roundworm of humans. *Enterobius vermicularis*, known as the human pinworm or seatworm, causes enterobiasis or oxyuriasis. *Strongyloides*, the threadworm, causes strongyloidiasis. *Ancylostoma*, *Ascaris*, and *Strongyloides* reside as adults in the small intestine of their natural hosts, whereas *E. vermicularis* colonizes the cecum and appendix.

Occupational Infections

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

Laboratory-acquired infections with these nematodes can be asymptomatic, or can present with a range of clinical manifestations dependent upon the species and their location in host. Infection with hookworm of animal origin can result in cutaneous larva migrans or creeping eruption of the skin. Infection with *A. lumbricoides* may produce cough, fever, and pneumonitis as larvae migrate through the lung, followed by abdominal cramps and diarrhea or constipation from adult worms in the intestine. Infection with *E. vermicularis* usually causes perianal pruritis, with intense itching. Infection with animal *Strongyloides* spp. may induce cutaneous larva migrans.

Natural Modes of Infection

Ancylostoma infection in dogs and cats is endemic worldwide. Human infection occurs through penetration of the skin. Cutaneous larva migrans or creeping eruption occurs when infective larvae of animal hookworms, typically dog and

cat hookworms, penetrate the skin and begin wandering. *Ancylostoma* larvae can also cause infection if ingested. These larvae do not typically reach the intestinal tract, although *A. caninum* has on rare occasions developed into non-gravid adult worms in the human gut.

Ascaris lumbricoides infection is endemic in tropical and subtropical regions of the world. Infection occurs following accidental ingestion of infective eggs. Unembryonated eggs passed in the stool require two to three weeks to become infectious, and *Ascaris* eggs are very hardy in the environment.

Enterobius vermicularis occurs worldwide, although 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 infective eggs, most often on contaminated fingers following scratching of the perianal skin. Eggs passed by female worms are not immediately infective, but only require several hours' incubation to become fully infectious. Infection with this worm is relatively short (60 days on average), and reinfection is required to maintain an infection.

Strongyloides infection in animals is endemic worldwide. People become infected with animal *Strongyloides* when infective, filariform larvae penetrate the skin, and can develop cutaneous creeping eruption (larva currens).

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 one day to several weeks. Ingestion of the infective eggs or skin penetration by infective larvae are the primary hazards to laboratory staff and animal care personnel. Development of hypersensitivity is common in laboratory personnel with frequent exposure to aerosolized antigens of ascarids.

Ascarid eggs are sticky, and special care should be taken to ensure thorough cleaning of contaminated surfaces and equipment. Caution should be used even when working with formalin-fixed stool samples because ascarid eggs can remain viable and continue to develop to the infective stage in formalin.[8]

Working with infective eggs of other ascarids, such as *Toxocara* and *Baylisascaris*, poses significant risk because of the potential for visceral migration of larvae, including invasion of the eyes and central nervous system. *Strongyloides stercoralis* is of particular concern to immuno-suppressed persons because potentially life-threatening systemic hyperinfection can occur. Lugol's iodine kills infective larvae and should be sprayed onto skin or laboratory surfaces that are contaminated accidentally. The larvae of *Trichinella* in fresh or digested tissue could cause infection if accidentally ingested. Arthropods infected with filarial parasites pose a potential hazard to laboratory personnel.

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

Special Issues

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

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

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Section VIII-D: Rickettsial Agents

Coxiella burnetii

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

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

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

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

Special Issues

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

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

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

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

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

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

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

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

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

Special Issues

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

Select Agent *R. prowazekii* and *R. rickettsii* are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

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

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Section VIII-E: Viral Agents

Hantaviruses

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

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

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

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

Special Issues

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

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

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

Occupational Infections

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

Natural Modes of Infection

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

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

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

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

Hepatitis A Virus, Hepatitis E Virus

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

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

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

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

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

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

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

Occupational Infections

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

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

Laboratory Safety and Containment Recommendations

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

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

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

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

Special Issues

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

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

Herpesvirus Simiae (Cerocopithecine Herpesvirus I, Herpes B Virus)

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

Occupational Infections

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

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

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

Natural Modes of Infection

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

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

Laboratory Safety and Containment Recommendations

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

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

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

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

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

Special Issues

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

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

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

Human Herpes Virus

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

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

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

Occupational Infections

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

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

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

Natural Modes of Infection

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

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

Laboratory Safety and Containment Recommendations

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

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

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

Special Issues

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

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

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

Influenza

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

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

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

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

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

Non-Contemporary Human Influenza (H2N2) Strains

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

1918 Influenza Strain

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

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

Highly Pathogenic Avian Influenza (HPAI)

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

Other Influenza Recombinant or Reassortant Viruses

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

- The gene constellation used.

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

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

Special Issues

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

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

Select Agent Strains of HPAI and 1918 influenza virus are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

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

Lymphocytic Choriomeningitis Virus

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

Occupational Infections

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

Natural Modes of Infection

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

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

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

Laboratory Safety and Containment Recommendations

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

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

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

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

Special Issues

Vaccines Vaccines are not available for use in humans.

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

Poliovirus

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

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

Occupational Infections

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

Natural Modes of Infection

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

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

Laboratory Safety and Containment Recommendations

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

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

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

Special Issues

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

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

Poxviruses

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

Occupational Infections

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

Natural Modes of Infection

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

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

Laboratory Safety and Containment Recommendations

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

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

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

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

Special Issues

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

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

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

Rabies Virus (and related lyssaviruses)

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

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

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

Special Issues

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

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

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

Occupational Infections

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

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

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

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

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

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

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

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

Special Issues

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

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

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

Severe Acute Respiratory Syndrome (SARS) Coronavirus

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

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

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

Occupational Infections

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

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

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

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

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

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

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

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

Special Issues

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

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

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

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

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

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Section VIII-F: Arboviruses and Related Zoonotic Viruses

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

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

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

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

These recommendations were made in August 2005; requirements for biosafety, shipping, and select agent registration can change. Please be sure to confirm the requirements with the appropriate Federal agency. If the pathogen of interest is one listed in Appendix D, contact the USDA for additional biosafety requirements. USDA guidance may supersede the information found in this Chapter.

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

Some commonly used vaccine strains for which attenuation has been firmly established are recognized by SALS. These vaccine strains may be handled safely at BSL-2 (Table 5). The agents in Table 4 and 5 may require permits from USDA/DOC/DHHS.

Table 4. Explanation of Symbols Used in Table 6 to Define Basis for Assignment of Viruses to Biosafety Levels

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

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

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

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

Viruses with BSL-2 Containment Recommended

The recommendation for conducting work with the viruses listed in Table 6 at BSL-2 are based on the existence of historical laboratory experience adequate to assess the risks when working with this group of viruses. This indicates a) no overt laboratory-associated infections are reported, b) infections resulted from exposures other than by infectious aerosols, or c) if disease from aerosol exposure is documented, it is uncommon.

Laboratory Safety and Containment Recommendations

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

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

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

Viruses with BSL-3 Containment Recommended

The recommendations for viruses listed in Table 6 that require BSL-3 containment are based on multiple criteria. SALS considered the laboratory experience for some viruses to be inadequate to assess risk, regardless of the available information regarding disease severity. In some cases, SALS recorded overt LAI transmitted by the aerosol route in the absence or non-use of protective vaccines, and considered that the natural disease in humans is potentially severe, life threatening, or causes residual damage.[1] Arboviruses also were classified as requiring BSL-3 containment if they caused diseases in domestic animals in countries outside of the United States.

Laboratory Safety and Containment Recommendations

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

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

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

Junin virus has been reclassified to BSL-3, provided that all at-risk personnel are immunized and the laboratory is equipped with HEPA-filtered exhaust. SALS also has reclassified Central European tick-borne encephalitis (CETBE) viruses to BSL-3, provided all at-risk personnel are immunized. CETBE is not a registered name in *The International Catalogue of Arboviruses* (1985). Until the registration issue is resolved taxonomically, CETBE refers to the following group of very closely related, if not essentially identical, tick-borne flaviviruses isolated from Czechoslovakia, Finland and Russia: Absettarov, Hanzalova, Hypr, and Kumlinge viruses. While there is a vaccine available that confers immunity to the CETBE group of genetically (>98%) homogeneous viruses, the efficacy of this vaccine against Russian spring-summer encephalitis (RSSE) virus infections has not been established. Thus, the CETBE group of viruses has been reclassified as BSL-3 when personnel are immunized with CETBE vaccine, while RSSE remains classified as BSL-4. It should be noted that CETBE viruses are currently listed as select agents and require special security and permitting considerations. (See Appendix F.)

Investigational vaccines for eastern equine encephalomyelitis (EEE) virus, Venezuelan equine encephalitis (VEE), western equine encephalomyelitis (WEE) virus, and Rift Valley fever viruses (RVFV), may be available in limited quantities and administered on-site at the Special Immunization Program of USAMRIID, located at Ft. Detrick, Frederick, MD. Details are available at the end of this section.

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

If the investigational vaccine is contraindicated, does not provide acceptable reliability for producing an immune response, or laboratory personnel refuse vaccination, the use of appropriate personal protective equipment may provide an alternative. Respiratory protection, such as use of a PAPR, should be considered in areas using organisms with a well-established risk of aerosol infections in the laboratory, such as VEE viruses.

Any respiratory protection equipment must be provided in accordance with the institution's respiratory protection program. Other degrees of respiratory protection may be warranted based on an assessment of risk as defined in Chapter 2 of this manual. All personnel in a laboratory with the infectious agent must use comparable personal protective equipment that meets or exceeds the requirements, even if they are not working with the organism. Sharps precautions as described under BSL-2 and BSL-3 requirements must be continually and strictly reinforced, regardless of whether investigational vaccines are used.

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

Enhanced BSL-3 Containment

Situations may arise for which enhancements to BSL-3 practices and equipment are required; for example, when a BSL-3 laboratory performs diagnostic testing on specimens from patients with hemorrhagic fevers thought to be due to dengue or yellow fever viruses. When the origin of these specimens is Africa, the Middle East, or South America, such specimens might contain etiologic agents, such as arenaviruses, filoviruses, or other viruses that are usually manipulated in a BSL-4

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

Viruses with BSL-4 Containment Recommended

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

Laboratory Safety and Containment Recommendations

The infectious agents may be present in blood, urine, respiratory and throat secretions, semen, and other fluids and tissues from human or animal hosts, and in arthropods, rodents, and NHPs. Respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and accidental parenteral inoculation are the primary hazards to laboratory or animal care personnel.[3,4]

BSL-4 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious materials of human, animal, or arthropod origin. Clinical specimens from persons suspected of being infected with one of the agents listed in this summary should be submitted to a laboratory with a BSL-4 maximum containment facility.[5]

Dealing with Unknown Arboviruses

The ACAV has published reports documenting laboratory workers who acquired arbovirus infections during the course of their duties.[6] In the first such document, it was recognized that these laboratory infections typically occurred by unnatural routes such as percutaneous or aerosol exposure, that “lab adapted” strains were still pathogenic for humans, and that as more laboratories worked with newly identified agents, the frequency of laboratory-acquired infections was increasing. Therefore, to assess the risk of these viruses and provide safety guidelines to those working with them, ACAV appointed SALS to evaluate the hazards of working with arboviruses in the laboratory setting.[7,8]

The SALS committee made a series of recommendations, published in 1980, describing four levels of laboratory practices and containment guidelines that were progressively more restrictive. These levels were determined after widely-distributed surveys evaluated numerous criteria for each particular virus including: 1) past occurrence of laboratory-acquired infections correlated with facilities and practices used; 2) volume of work performed as a measure of

potential exposure risk; 3) immune status of laboratory personnel; 4) incidence and severity of naturally-acquired infections in adults; and 5) incidence of disease in animals outside the United States (to assess import risk).

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

One criterion for a newly identified arbovirus is a thorough description of how the virus will be handled and investigated. For example, experiments involving pure genetic analysis could be handled differently than those where the virus will be put into animals or arthropods.[9] Additionally, an individual risk assessment should consider the fact that not all strains of a particular virus exhibit the same degree of pathogenicity or transmissibility. While variable pathogenicity occurs frequently with naturally identified strains, it is of particular note for strains that are modified in the laboratory. It may be tempting to assign biosafety levels to hybrid or chimeric strains based on the parental types but due to possible altered biohazard potential, assignment to a different biosafety level may be justified.[10] A clear description of the strains involved should accompany any risk assessment.

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

Chimeric Viruses

The ability to construct cDNA clones encoding a complete RNA viral genome has led to the generation of recombinant viruses containing a mixture of genes from two or more different viruses. Chimeric, full-length viruses and truncated replicons have been constructed from numerous alphaviruses and flaviviruses. For example, alphavirus replicons encoding foreign genes have been used

widely as immunogens against bunyavirus, filovirus, arenavirus, and other antigens. These replicons have been safe and usually immunogenic in rodent hosts leading to their development as candidate human vaccines against several virus groups including retroviruses.[11-14]

Because chimeric viruses contain portions of multiple viruses, the IBC, in conjunction with the biosafety officer and the researchers, must conduct a risk assessment that, in addition to standard criteria, includes specific elements that need to be considered before assigning appropriate biosafety levels and containment practices. These elements include: 1) the ability of the chimeric virus to replicate in cell culture and animal model systems in comparison with its parental strains;[15] 2) altered virulence characteristics or attenuation compared with the parental viruses in animal models;[16] 3) virulence or attenuation patterns by intracranial routes using large doses for agents affecting the CNS;[17,18] and 4) demonstration of lack of reversion to virulence or parental phenotype.

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

Chimeric viruses may have some safety features not associated with parental viruses. For example, they are generated from genetically stable cDNA clones without the need for animal or cell culture passage. This minimizes the possibility of mutations that could alter virulence properties. Because some chimeric strains incorporate genomic segments lacking gene regions or genetic elements critical for virulence, there may be limited possibility of laboratory recombination to generate strains exhibiting wild-type virulence.

Ongoing surveillance and laboratory studies suggest that many arboviruses continue to be a risk to human and animal populations. The attenuation of all chimeric strains should be verified using the most rigorouscontainment requirements of the parental strains. The local IBC should evaluate containment recommendations for each chimeric virus on a case-by-case basis, using virulence data from an appropriate animal model. Additional guidance from the NIH Office of Biotechnology Activities and/or the Recombinant DNA Advisory Committee (RAC) may be necessary.

West Nile Virus (WNV)

WNV has emerged in recent years in temperate regions of Europe and North America, presenting a threat to public and animal health. This virus belongs to the family *Flaviviridae* and the genus *Flavivirus*, Japanese encephalitis virus antigenic complex. The complex currently includes Alfuy, Cacipacore, Japanese encephalitis, Koutango, Kunjin, Murray Valley encephalitis, St. Louis encephalitis,

Rocio, Stratford, Usutu, West Nile, and Yaounde viruses. Flaviviruses share a common size (40-60nm), symmetry (enveloped, icosahedral nucleocapsid), nucleic acid (positive-sense, single stranded RNA approximately 10,000-11,000 bases) and virus morphology. The virus was first isolated from a febrile adult woman in the West Nile District of Uganda in 1937.[20] The ecology was characterized in Egypt in the 1950s; equine disease was first noted in Egypt and France in the early 1960s.[21,22] It first appeared in North America in 1999 as encephalitis reported in humans and horses.[23] The virus has been detected in Africa, Europe, the Middle East, west and central Asia, Oceania (subtype Kunjin virus), and most recently, North America.

Occupational Infections

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

Natural Modes of Infections

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

Laboratory Safety and Containment Recommendations

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

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

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

Special Issues

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

Eastern Equine Encephalitis (EEE) Virus, Venezuelan Equine Encephalitis (VEE) Virus, and Western Equine Encephalitis (WEE) Virus

VEE, EEE, and WEE viruses are members of the genus *Alphavirus* in the family *Togaviridae*. They are small, enveloped viruses with a genome consisting of a single strand of positive-sense RNA. All three viruses can cause encephalitis often accompanied by long-term neurological sequelae. Incubation period ranges from 1-10 days and the duration of acute illness is typically days to weeks depending upon severity of illness. Although not the natural route of transmission, the viruses are highly infectious by the aerosol route; laboratory acquired infections have been documented.[25]

Occupational Infections

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

Natural Modes of Infection

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

EEE virus occurs in focal locations along the eastern seaboard, the Gulf Coast and some inland Midwestern locations of the United States, in Canada, some Caribbean Islands, and Central and South America.[26] Small outbreaks of human disease have occurred in the United States, the Dominican Republic, Cuba, and Jamaica. In the United States, equine epizootics are common occurrences during the summer in coastal regions bordering the Atlantic and Gulf of Mexico, in other eastern and Midwestern states, and as far north as Quebec, Ontario, and Alberta in Canada.

In Central and South America, focal outbreaks due to VEE virus occur periodically with rare large regional epizootics involving thousands of equine cases and deaths in predominantly rural settings. These epizootic/epidemic viruses are theorized to emerge periodically from mutations occurring in the continuously circulating enzootic VEE viruses in northern South America. The classical epizootic varieties of the virus are not present in the United States. An enzootic subtype, Everglades virus (VEE antigenic complex subtype II virus), exists naturally in southern Florida, while endemic foci of Bijou Bridge virus (VEE antigenic complex subtype III-B virus), have been described in the western United States.[27]

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

Laboratory Safety and Containment Recommendations

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

Diagnostic and research activities involving clinical material, infectious cultures, and infected animals or arthropods should be performed under BSL-3 practices, containment equipment, and facilities. Due to the high risk of aerosol infection, additional personal protective equipment, including respiratory protection, should be considered for non-immune personnel. Animal work with VEE virus, EEE virus and WEE virus should be performed under ABSL-3 conditions. HEPA filtration is required on the exhaust system of laboratory and animal facilities using VEE virus.

Special Issues

Vaccines Two strains of VEE virus (TC-83 and V3526) are highly attenuated in vertebrate studies and have been either exempted (strain TC-83) or excluded (strain V3526) from select agent regulations. Because of the low level of pathogenicity, these strains may be safely handled under BSL-2 conditions without vaccination or additional personal protective equipment.

Investigational vaccine protocols have been developed to immunize at-risk laboratory or field personnel against these alphaviruses, however, the vaccines are available only on a limited basis and may be contraindicated for some personnel. Therefore, additional personal protective equipment may be warranted in lieu of vaccination. For personnel who have no neutralizing antibody titer (either by previous vaccination or natural infection), additional respiratory protection is recommended for all procedures.

Select Agent VEE virus and EEE virus are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

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

Rift Valley Fever Virus (RVFV)

RVFV was first isolated in Kenya in 1936 and subsequently shown to be endemically present in almost all areas of sub-Saharan Africa.[28] In periods of heavy rainfall, large epizootics occur involving primarily sheep, cattle, and human disease, although many other species are infected. The primordial vertebrate reservoir is unknown, but the introduction of large herds of highly susceptible domestic breeds in the last few decades has provided a substrate for massive virus amplification. The virus has been introduced into Egypt, Saudi Arabia, and Yemen and caused epizootics and epidemics in those countries. The largest of these was in 1977 to 1979 in Egypt with many thousands of human cases and 610 reported deaths.[29]

Most human infections are symptomatic and the most common syndrome consists of fever, myalgia, malaise, anorexia, and other non-specific symptoms. Recovery within one to two weeks is usual but hemorrhagic fever, encephalitis, or retinitis also occurs. Hemorrhagic fever develops as the primary illness proceeds and is characterized by disseminated intravascular coagulation and hepatitis. Perhaps 2% of cases will develop this complication and the mortality is high. Encephalitis follows an apparent recovery in <1% of cases and results in a substantial mortality and sequelae. Retinal vasculitis occurs in convalescence of a substantial but not precisely known proportion of cases. The retinal lesions are often macular and permanent, leading to substantial loss of visual acuity.

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

Occupational Infections

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

There have been 47 reported laboratory infections; before modern containment and vaccination became available virtually every laboratory that began work with the virus suffered infections suggestive of aerosol transmission.[30,31]

Natural Modes of Infection

Field studies show RVFV to be transmitted predominantly by mosquitoes, although other arthropods may be infected and transmit. Mechanical transmission also has been documented in the laboratory. Floodwater *Aedes* species are the primary vector and transovarial transmission is an important part of the maintenance cycle.[32] However, many different mosquito species are implicated in horizontal transmission in field studies, and laboratory studies have shown a large number of mosquito species worldwide to be competent vectors, including North American mosquitoes.

It is currently believed that the virus passes dry seasons in the ova of flood-water *Aedes* mosquitoes. Rain allows infectious mosquitoes to emerge and feed on vertebrates. Several mosquito species can be responsible for horizontal spread, particularly in epizootic/epidemic situations. The vertebrate amplifiers are usually sheep and cattle, with two caveats; as yet undefined native African vertebrate amplifier is thought to exist and very high viremias in humans are thought to play some role in viral amplifications.[33]

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

Laboratory Safety and Containment Recommendations

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

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

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

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

Special Issues

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[b] Tentative placement in the genus.

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

[d] Alternate name for Ganjam virus.

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

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

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Section VIII-G: Toxin Agents

Botulinum Neurotoxin

Seven immunologically distinct serotypes of Botulinum neurotoxin (BoNT) have been isolated (A, B, C1, D, E, F and G). Each BoNT holotoxin is a disulfide-bonded heterodimer composed of a zinc metallo-protease "light chain" (approximately 50 kD) and a receptor binding "heavy chain" (approximately 100 kD). The heavy chain enhances cell binding and translocation of the catalytic light chain across the vesicular membrane.[1] There are also a number of important accessory proteins that can stabilize the natural toxin complex in biological systems or in buffer.

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

Diagnosis of Laboratory Exposures

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

Laboratory Safety and Containment Recommendations

Solutions of sodium hypochlorite (0.1%) or sodium hydroxide (0.1N) readily inactivate the toxin and are recommended for decontamination of work surfaces and for spills. Additional considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Because neurotoxin producing Clostridia species requires an anaerobic environment for growth and it is essentially not transmissible among individuals, exposure to pre-formed BoNT is the primary concern for laboratory workers. Two of the most significant hazards in working with BoNT or growing neurotoxin producing Clostridia species cultures are unintentional aerosol generation, especially during centrifugation, and accidental needle-stick. Although BoNT does not penetrate intact skin,

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

Workers in diagnostic laboratories should be aware that neurotoxin producing Clostridia species or its spores can be stable for weeks or longer in a variety of food products, clinical samples (e.g., serum, feces) and environmental samples (e.g., soil). Stability of the toxin itself will depend upon the sterility, temperature, pH and ionic strength of the sample matrix, but useful comparative data are available from the food industry. BoNT retains its activity for long periods (at least 6-12 months) in a variety of frozen foods, especially under acidic conditions (pH 4.5-5.0) and/or high ionic strength, but the toxin is readily inactivated by heating.[5]

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

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

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

Special Issues

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

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

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

Staphylococcal Enterotoxins (SE)

SE are a group of closely related extracellular protein toxins of 23 to 29 kD molecular weight that are produced by distinct gene clusters found in a wide variety of *S. aureus* strains.[8,9] SE belong to a large family of homologous pyrogenic exotoxins from staphylococci, streptococci and mycoplasma which are capable of causing a range of illnesses in man through pathological amplification of the normal T-cell receptor response, cytokine/lymphokine release, immunosuppression and endotoxic shock.[9,10]

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

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

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

Diagnosis of Laboratory Exposures

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

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

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

Laboratory confirmation of intoxication includes SE detection by immunoassay of environmental and clinical samples, and gene amplification to detect staphylococcal genes in environmental samples. SE may be undetectable in the serum at the time symptoms occur; nevertheless, a serum specimen should be drawn as early as possible after exposure. Data from animal studies suggest the presence of SE in the serum or urine is transient. Respiratory secretions and nasal swabs may demonstrate the toxin early (within 24 h of inhalation exposure). Evaluation of neutralizing antibody titers in acute and convalescent sera of exposed individuals can be undertaken, but may yield false positives resulting from pre-existing antibodies produced in response to natural SE exposure.

Laboratory Safety and Containment Recommendations

General considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Accidental ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes are believed to be the primary hazards of SE for laboratory and animal-care personnel. SE are relatively stable, monomeric proteins, readily soluble in water, and resistant to proteolytic degradation and temperature fluctuations. The physical/chemical stability of SE suggests that additional care must be taken by laboratory workers to avoid exposure to residual toxin that may persist in the environment.

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

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

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

BSL-2 practices and containment equipment and facilities should be used when handling SE or potentially contaminated material. Because SE is highly active by the oral or ocular exposure route, the use of a laboratory coat, gloves and safety glasses is mandatory when handling toxin or toxin-contaminated solutions. Frequent and careful hand-washing and laboratory decontamination should be strictly enforced when working with SE. Depending upon a risk assessment of the laboratory operation, the use of a disposable face mask may be required to avoid accidental ingestion.

BSL-3 facilities, equipment, and practices are indicated for activities with a high potential for aerosol or droplet production and those involving the use of large quantities of SE.

Special Issues

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

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

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

Ricin Toxin

Ricin is produced in maturing seeds of the castor bean, *Ricinus communis,* which has been recognized for centuries as a highly poisonous plant for humans and livestock.[20] Ricin belongs to a family of ribosome inactivating proteins from plants, including abrin, modeccin, and viscumin, that share a similar overall structure and mechanism of action.[21] The ricin holotoxin is a disulfide-bonded heterodimer composed of an A-chain (approximately 34 kD polypeptide) and a B-chain (approximately 32 kD). The A-chain is an N-glycosidase enzyme and a potent inhibitor of protein synthesis, whereas the B-chain is a relatively non-toxic lectin that facilitates toxin binding and internalization to target cells.[20]

Ricin is much less toxic by weight than is BoNT or SE, and published case reports suggest that intramuscular or gastric ingestion of ricin is rarely fatal in adults.[22] Animal studies and human poisonings suggest that the effects of ricin

depend upon the route of exposure, with inhalation and intravenous exposure being the most toxic. In laboratory mice, for example, the LD_{50} by intravenous injection is about 5 µg/kg, whereas it is 20 mg/kg by intragasteric route.[23,24] The ricin aerosol LD_{50} for NHP is estimated to be 10-15 µg/kg.[17] The human lethal dose has not been established rigorously, but may be as low as 1-5 mg of ricin by injection 25 or by the aerosol route (extropolation from two species of NGP).

Diagnosis of Laboratory Exposures

The primary diagnosis is through clinical manifestations that vary greatly depending upon the route of exposure. Following inhalation exposure of NHP, there is typically a latency period of 24-72 h that may be characterized by loss of appetite and listlessness. The latency period progresses rapidly to severe pulmonary distress, depending upon the exposure level. Most of the pathology occurs in the lung and upper respiratory tract, including inflammation, bloody sputum, and pulmonary edema. Toxicity from ricin inhalation would be expected to progress despite treatment with antibiotics, as opposed to an infectious process. There would be no mediastinitis as seen with inhalation anthrax. Ricin patients would not be expected to plateau clinically as occurs after inhalation of SEB.

Gastric ingestion of ricin causes nausea, vomiting, diarrhea, abdominal cramps and dehydration. Initial symptoms may appear more rapidly following gastric ingestion (1-5 h), but generally require exposure to much higher levels of toxin compared with the inhalation route. Following intramuscular injection of ricin, symptoms may persist for days and include nausea, vomiting, anorexia, and high fever. The site of ricin injection typically shows signs of inflammation with marked swelling and induration. One case of poisoning by ricin injection resulted in fever, vomiting, irregular blood pressure, and death by vascular collapse after a period of several days; it is unclear in this case if the toxin was deposited intramuscularly or in the bloodstream.[25]

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

Laboratory Safety and Containment Recommendations

General considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Precautions should be extended to handling potentially contaminated clinical, diagnostic and post-mortem samples because

ricin may retain toxicity in the lesion fluids, respiratory secretions, or unfixed tissues of exposed animals.

When the ricin A-chain is separated from the B-chain and administered parenterally to animals, its toxicity is diminished by >1,000-fold compared with ricin holotoxin.[26] However, purified preparations of natural ricin A-chain or B-chain, as well as crude extracts from castor beans, should be handled as if contaminated by ricin until proven otherwise by bioassay.

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

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

Special Issues

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

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

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

Selected Low Molecular Weight (LMW) Toxins

LMW toxins comprise a structurally and functionally diverse class of natural poisons, ranging in size from several hundred to a few thousand daltons, that includes complex organic structures, as well as disulfide cross-linked and cyclic polypeptides. Tremendous structural diversity may occur within a particular type of LMW toxin, often resulting in incomplete toxicological or pharmacological characterization of minor isoforms. Grouping LMW toxins together has primarily been a means of distinguishing them from protein toxins with respect to key biophysical characteristics. Compared with proteins, the LMW toxins are of smaller size, which alters their filtration and biodistribution properties, are

generally more stable and persistent in the environment, and may exhibit poor water-solubility necessitating the use of organic solvent; these characteristics pose special challenges for safe handling, containment, and decontamination of LMW toxins within the laboratory.

The set of LMW toxins selected for discussion herein are employed routinely as laboratory reagents, and/or have been designated as potential public health threats by the CDC, including: T-2 mycotoxin produced by *Fusarium* fungi;[27,28] saxitoxin and related paralytic shellfish poisons produced by dinoflagellates of the *Gonyaulax* family;[29] tetrodotoxin from a number of marine animals,[30] brevetoxin from the dinoflagellate *Ptychodiscus brevis*;[31] palytoxin from marine coelenterates belonging to the genus *Palythoa*,[32] polypeptide conotoxins α-GI (includes GIA) and α-MI from the *Conus* genus of gastropod mollusks;[33] and the monocyclic polypeptide, microcystin-LR from freshwater cyanobacteria *Microcystis aeruginosa*.[34]

Trichothecene mycotoxins comprise a broad class of structurally complex, non-volatile sesquiterpene compounds that are potent inhibitors of protein synthesis.[27,28] Mycotoxin exposure occurs by consumption of moldy grains, and at least one of these toxins, designated "T-2," has been implicated as a potential biological warfare agent.[27] T-2 is a lipid-soluble molecule that can be absorbed into the body rapidly through exposed mucosal surfaces.[35] Toxic effects are most pronounced in metabolically active target organs and include emesis, diarrhea, weight loss, nervous disorder, cardiovascular alterations, immunodepression, hemostatic derangement, bone marrow damage, skin toxicity, decreased reproductive capacity, and death.[27] The LD_{50} for T-2 in laboratory animals ranges from 0.2 to 10 mg/kg, depending on the route of exposure, with aerosol toxicity estimated to be 20 to 50 times greater than parenteral exposure.[17,27] Of special note, T-2 is a potent vesicant capable of directly damaging skin or corneas. Skin lesions, including frank blisters, have been observed in animals with local, topical application of 50 to 100 ng of toxin.[27,35]

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

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

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

Palytoxin is a structurally complex, articulated fatty acid associated with soft coral *Palythoa vestitus* that is capable of binding and converting the essential cellular Na+/K+ pump into a non-selective cation channel.[32,39] Palytoxin is among the most potent coronary vasoconstrictors known, killing animals within minutes by cutting off oxygen to the myocardium.[40] The LD_{50} for intravenous administration ranges from 0.025 to 0.45 µg/kg in different species of laboratory animals.[40] Palytoxin is lethal by several parenteral routes, but is about 200-fold less toxic if administered to the alimentary tract (oral or rectal) compared with intravenous administration.[40] Palytoxin disrupts normal corneal function and causes irreversible blindness at topically applied levels of approximately 400 ng/kg, despite extensive rinsing after ocular instillation.[40]

Conotoxins are polypeptides, typically 10-30 amino acids long and stabilized by distinct patterns of disulfide bonds, that have been isolated from the toxic venom of marine snails and shown to be neurologically active or toxic in mammals.[33] Of the estimated >105 different polypeptides (conopeptides) present in venom of over 500 known species of *Conus*, only a few have been rigorously tested for animal toxicity. Of the isolated conotoxin subtypes that have been analyzed, at least two post-synaptic paralytic toxins, designated α-GI (includes GIA) and α-MI, have been reported to be toxic in laboratory mice with LD_{50} values in the range of 10-100 µg/kg depending upon the species and route of exposure.

Workers should be aware, however, that human toxicity of whole or partially fractionated *Conus* venom, as well as synthetic combinations of isolated conotoxins, may exceed that of individual components. For example, untreated cases of human poisoning with venom of C*. geographus* result in an approximately 70% fatality rate, probably as a result of the presence of mixtures of various α- and µ-conotoxins with common or synergistic biological targets.[33,41] The α-conotoxins act as potent nicotinic antagonists and the µ-conotoxins block the sodium channel.[33] Symptoms of envenomation depend upon the *Conus* species involved, generally occur rapidly after exposure (minutes), and range from severe pain to spreading numbness.[42] Severe intoxication results in muscle paralysis, blurred or double vision, difficulty breathing and swallowing, and respiratory or cardiovascular collapse.[42]

Microcystins (also called cyanoginosins) are monocyclic heptapeptides composed of specific combinations of L-, and D-amino acids, some with uncommon side chain structures, that are produced by various freshwater cyanobacteria.[43] The toxins are potent inhibitors of liver protein phosphatase type 1 and are capable of causing massive hepatic hemorrhage and death.[43]

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

Diagnosis of Laboratory Exposures

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

Specific diagnosis of LMW toxins in the form of a rapid diagnostic test is not presently available in the field. Serum and urine should be collected for testing at specialized reference laboratories by methods including antigen detection, receptor-binding assays, or liquid chromatographic analyses of metabolites. Metabolites of several marine toxins, including saxitoxin, tetrodotoxin, and brevetoxins, are well-studied as part of routine regulation of food supplies.[36] Likewise, T-2 mycotoxin absorption and biodistribution has been studied, and its metabolites can be detected as late as 28 days after exposure.[27] Pathologic specimens include blood, urine, lung, liver, and stomach contents. Environmental and clinical samples can be tested using a gas liquid chromatography-mass spectrometry technique.

Laboratory Safety and Containment Recommendations

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

In addition to their high toxicity, the physical/chemical stability of the LMW toxins contribute to the risks involved in handling them in the laboratory environment. Unlike many protein toxins, the LMW toxins can contaminate surfaces as a stable, dry film that may pose an essentially indefinite contact

threat to laboratory workers. Special emphasis, therefore, must be placed upon proper decontamination of work surfaces and equipment.[45]

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

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

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

Special Issues

Vaccines No approved vaccines are currently available for human use. Experimental therapeutics for LMW toxins have been reviewed.[46]

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

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

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Section VIII-H: Prion Diseases

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

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

Occupational Infections

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

Natural Modes of Infection

The recognized diseases caused by prions are listed under Table 7 (human diseases) and Table 8 (animal diseases). The only clear risk factor for disease transmission is the consumption of infected tissues such as human brain in the case of kuru, and meat including nervous tissue in the case of bovine spongiform encephalopathy and related diseases such as feline spongiform encephalopathy. It is also possible to acquire certain diseases such as familial CJD by inheritance through the germ line.

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

Table 7. The Human Prion Diseases

Disease	Abbreviation	Mechanism of Pathogenesis
Kuru		Infection through ritualistic cannibalism
Creutzfeldt-Jakob disease	CJD	Unknown mechanism
Sporadic CJD	sCJD	Unknown mechanism; possibly somatic mutation or spontaneous conversion of PrP^{c} to PrP^{Sc}
Variant CJD	vCJD	Infection presumably from consumption of BSE-contaminated cattle products and secondary bloodborne transmission
Familial CJD	fCJD	Germline mutations in PrP gene
Latrogenic CJD	iCJD	Infection from contaminated corneal and dural grafts, pituitary hormone, or neurosurgical equipment
Gerstmann-Sträussler-Scheinker syndrome	GSS	Germline mutations in PrP gene
Fatal familial insomnia	FFI	Germline mutations in PrP gene

Table 8. The Animal Prion Diseases

Disease	Abbreviation	Natural Host	Mechanism of Pathogenesis
Scrapie		Sheep, goats, mouflon	Infection in genetically susceptible sheep
Bovine spongiform encephalopathy	BSE	Cattle	Infection with prion-contaminated feedstuffs
Chronic wasting disease	CWD	Mule, deer, white-tailed deer, Rocky Mountain elk	Unknown mechanism; possibly from direct animal contact or indirectly from contaminated feed and water sources
Exotic ungulate encephalopathy	EUE	Nyala, greater kudu and oryx	Infection with BSE-contaminated feedstuffs
Feline spongiform encephalopathy	FSE	Domestic and wild cats in captivity	Infection with BSE-contaminated feedstuffs
Transmissible mink encephal-opathy	TME	Mink (farm raised	Infection with prion-contaminated feedstuffs

Laboratory Safety and Containment Recommendations

In the laboratory setting prions from human tissue and human prions propagated in animals should be manipulated at BSL-2. BSE prions can likewise be manipulated at BSL-2. Due to the high probability that BSE prions have been transmitted to humans, certain circumstances may require the use of BSL-3 facilities and practices. All other animal prions are manipulated at BSL-2. However, when a prion from one species is inoculated into another the resultant infected animal should be treated according to the guidelines applying to the source of the inoculum. Contact APHIS National Center for Import and Export at (301) 734-5960 for specific guidance.

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

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

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

Special Issues

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

The safest and most unambiguous method for ensuring that there is no risk of residual infectivity on contaminated instruments and other materials is to discard and destroy them by incineration.[6] Current recommendations for inactivation of prions on instruments and other materials are based on the use of sodium hypochlorite, NaOH, Environ LpH and the moist heat of autoclaving with combinations of heat and chemical being most effective (Table 9).[5,6]

Surgical Procedures Precautions for surgical procedures on patients diagnosed with prion disease are outlined in an infection control guideline for transmissible spongiform encephalopathies developed by a consultation convened by the WHO in 1999.[6] Sterilization of reusable surgical instruments and decontamination of surfaces should be performed in accordance with recommendations described by the CDC (*www.cdc.gov*) and the WHO infection control guidelines.[6] Table 9 summarizes the key recommendations for decontamination of reusable instruments and surfaces. Contaminated disposable instruments or materials should be incinerated at 1000° C or greater.[7]

Autopsies Routine autopsies and the processing of small amounts of formalin-fixed tissues containing human prions can safely be done using Standard Precautions.[8] The absence of any known effective treatment for prion disease demands caution. The highest concentrations of prions are in the central nervous system and its coverings. Based on animal studies, it is likely that prions are also found in spleen, thymus, lymph nodes, and intestine. The main precaution to be taken by laboratorians working with prion-infected or contaminated material is to avoid accidental puncture of the skin.[3] Persons handling contaminated specimens should wear cut-resistant gloves if possible. If accidental contamination of unbroken skin occurs, the area should be washed with detergent and abundant quantities of warm water (avoid scrubbing); brief exposure (1 minute to 1N NaOH or a 1:10 dilution of bleach) can be considered for maximum safety.[6] Additional guidance related to occupational injury are provided in the WHO infection control guidelines.[6] Unfixed samples of brain, spinal cord, and other tissues containing human prions should be processed with extreme care in a BSL-2 facility utilizing BSL-3 practices.

Bovine Spongiform Encephalopathy Although the eventual total number of variant CJD cases resulting from BSE transmission to humans is unknown, a review of the epidemiological data from the United Kingdom indicates that BSE transmission to humans is not efficient.[9] The most prudent approach is to study BSE prions at a minimum in a BSL-2 facility utilizing BSL-3 practices. When performing necropsies on large animals where there is an opportunity that the worker may be accidentally splashed or have contact with high-risk materials (e.g., spinal column, brain) personnel should wear full body coverage personal protective equipment (e.g., gloves, rear closing gown and face shield). Disposable plasticware, which can be discarded as a dry regulated medical waste, is highly recommended. Because the paraformaldehyde vaporization procedure does not diminish prion titers, BSCs must be decontaminated with 1N NaOH and rinsed with water. HEPA filters should be bagged out and incinerated. Although there is no evidence to suggest that aerosol transmission occurs in the natural disease, it is prudent to avoid the generation of aerosols or droplets during the manipulation of tissues or fluids and during the necropsy of experimental animals. It is further strongly recommended that impervious gloves be worn for activities that provide the opportunity for skin contact with infectious tissues and fluids.

Animal carcasses and other tissue waste can be disposed by incineration with a minimum secondary temperature of 1000°C (1832°F).[6] Pathological incinerators should maintain a primary chamber temperature in compliance with design and applicable state regulations, and employ good combustion practices. Medical waste incinerators should comply with applicable state and federal regulations.

The alkaline hydrolysis process, using a pressurized vessel that exposes the carcass or tissues to 1 N NaOH or KOH heated to 150°C, can be used as an alternative to incineration for the disposal of carcasses and tissue.[5,10] The process has been shown to completely inactive TSEs (301v agent used) when used for the recommended period.

Table 9. Tissue Preparation for Human CJD and Related Diseases

1.	Histology technicians wear gloves, apron, laboratory coat, and face protection.
2.	Adequate fixation of small tissue samples (e.g., biopsies) from a patient with suspected prion disease can be followed by post-fixation in 96% absolute formic acid for 30 minutes, followed by 45 hours in fresh 10% formalin.
3.	Liquid waste is collected in a 4L waste bottle initially containing 600 ml 6N NaOH.
4.	Gloves, embedding molds, and all handling materials are disposed s regulated medical waste.
5.	Tissue cassettes are processed manually to prevent contamination of tissue processors.
6.	Tissues are embedded in a disposable embedding mold. If used, forceps are decontaminated as in Table 10.
7.	In preparing sections, gloves are worn, section waste is collected and disposed in a regulated medical waste receptacle. The knife stage is wiped with 2N NaOH, and the knife used is discarded immediately in a "regulated medical waste sharps" receptacle. Slides are labeled with "CJD Precautions." The sectioned block is sealed with paraffin.
8.	Routine staining: a. slides are processed by hand; b. reagents are prepared in 100 ml disposable specimen cups; c. after placing the cover slip on, slides are decontaminated by soaking them for 1 hour in 2N NaOH; d. slides are labeled as "Infectious-CJD."
9.	Other suggestions: a. disposable specimen cups or slide mailers may be used for reagents; b. slides for immunocytochemistry may be processed in disposable Petri dishes; c. equipment is decontaminated as described above or disposed as regulated medical waste.

Handling and processing of tissues from patients with suspected prion disease The special characteristics of work with prions require particular attention to the facilities, equipment, policies, and procedures involved.[10] The related considerations outlined in Table 9 should be incorporated into the laboratory's risk management for this work.

Table 10. Prion Inactivation Methods for Reusable Instruments and Surfaces

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

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

Working Solutions 1 N NaOH equals 40 grams of NaOH per liter of water. Solution should be prepared daily. A stock solution of 10 N NaOH can be prepared and fresh 1:10 dilutions (1 part 10 N NaOH plus 9 parts water) used daily.

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

CAUTION: Above solutions are corrosive and require suitable personal protective equipment and proper secondary containment. These strong corrosive solutions require careful disposal in accordance with local regulations.

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

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