BMBL5
Containment
Recommendations

Bacterial Agents

Agent Name	Containment Recommendations	Select Agent
Bacillus anthracis	**BSL-3:** Production quantities, high concentrations of cultures, screening environmental samples (especially powders) from anthrax-contaminated locations, and for activities with a high potential for aerosol production. BSL-2: Clinical materials and diagnostic quantities of infectious cultures. ABSL-2: Rodent studies	*B. anthracis* is a **Select Agent**
Bordetella pertussis	**BSL-3:** Production operations. BSL-2: Known or potentially infectious clinical material and cultures. ABSL-2: Experimentally infected animals.	
Brucella species	**BSL-3** and A**BSL-3:** All manipulations of cultures of pathogenic *Brucella* spp. listed in this summary, and for experimental animal studies. **BSL-3:** Products of conception due to the high concentration of organisms per gram of tissue. BSL-2: Routine clinical specimens of human or animal origin.	*Brucella abortus, Brucella melitensis, and Brucella suis* are **Select Agents**
Burkholderia mallei	**BSL-3:** Infectious aerosols, droplets, centrifugation, handling infected animals, or when large quantities of the agent are produced. A**BSL-3:** *B. pseudomallei* and *B. mallei* are two closely related select agents that cause melioidosis and glanders, respectively. *B. mallei* causes natural glanders, a rare disease of equids (*1*), although it can also cause rare infections in humans (*2*). By contrast, the disease caused by *B. pseudomallei*, human melioidosis, is endemic to much of Southeast Asia, northern Australia, and other parts of the Tropics. BSL-2: Primary isolations from patient fluids or tissues in a BSC.	*B. mallei* is a **Select Agent**
Burkholderia pseudomallei	BSL-2: Clinical specimens of melioidosis and *B. pseudomallei* cultures, in BSC. **BSL-3:** Aerosols or droplets, production quantities should be confined to facilities with all pertinent primary containment against escape of aerosols. **ABSL-3**	*B. pseudomallei* is a **Select Agent**
Chlamydia psittaci (Chlamydophila psittaci), C. trachomatis, C. pneumoniae (Chlamydophila pneumoniae)	BSL-2: Clinical specimens, cultures of ocular or genital serovars (A through K) of *C. trachomatis* or *C. pneumoniae*. **BSL-3:** Necropsy of infected birds, tissues or cultures of *C. psittaci* strains of avian origin. BSL-2 with BSL-3 practices and BSC: Non-avian strains of *C. psittaci* **ABSL-3:** Infected caged birds. **BSL-3:** Clinical, culture specimens of LGV serovars (L1 through L3) of *C. trachomatis*. BSL-2 with BSL-3 practices and BSC: LGV serovars of *C. trachomatis* ABSL-2: Animals infected with genital serovars of *C. trachomatis* or *C. pneumoniae*. **BSL-3:** Any of these species with high potential for droplet or aerosol production, large quantities or concentrations.	
Neurotoxin producing Clostridia species Clostridium botulinum, and rare strains of C. baratii and C. butyricum,	**BSL-3**: Aerosol or droplet production, routine handling of larger quantities of the organism or of the toxin. ABSL-2: Diagnostic studies and titration of toxin.	Neurotoxin producing *Clostridia* species are **Select Agents**
Francisella tularensis	**BSL-3** and A**BSL-3:** Cultures (including preparatory work), animal necropsies, animal studies. Manipulation of reduced virulence strains at high concentrations.	*F. tularensis* is a **Select Agent**

	BSL-2: Clinical materials of human or animal origin. 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.	
Mycobacterium tuberculosis complex	**BSL-3:** Cultures of any of the subspecies of the *M. tuberculosis* complex, animal studies using NHP. **BSL-3:** Practices should include respiratory protection, implementation of procedures and equipment to prevent and contain aerosols. BSL-2: Non-aerosol-producing manipulations of clinical specimens; all aerosol-generating activities in a BSC. 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. ABSL-2: Animal studies using guinea pigs or mice.	
Neisseria gonorrhoeae	**BSL- 3:** High risk of aerosol or droplet production, production quantities, high concentrations. BSL-2: Clinical materials or cultures. ABSL-2	
Neisseria meningitidis	**BSL-3:** Droplet or aerosol production, production quantities, high concentrations. BSL-2: Specimens, cultures for *N. meningitidis* not associated with invasive disease may be handled with rigorous application of BSL-2 standard practices, special practices, and safety equipment. ABSL-2	
Salmonella Typhi	A**BSL-3** conditions may be considered for protocols involving aerosols. BSL-2: Strict compliance. ABSL-2	
Yersinia pestis	**BSL-3:** Droplet, aerosol production, large scale production or high concentrations. ABSL-2 or A**BSL-3** **BSL-3** facilities and arthropod containment level 3 practices for all with infected arthropods. BSL-2 clinical materials and cultures; necropsies in a BSC.	*Yersinia pestis* is an HHS **Select Agent**

Fungal Agents

Agent Name	Containment Recommendations	Select Agent
Blastomyces dermatitidis	**BSL-3:** Sporulating mold-form cultures identified as *B. dermatitidis* and soil or other environmental samples known or likely to contain infectious conidia. BSL-2: Clinical materials, animal tissues, yeast-form cultures. ABSL-2	
Coccidioides immitis and Coccidioides posadasii	**BSL-3:** Propagating, manipulating sporulating cultures. **ABSL-3:** Challenge via the intranasal or pulmonary route. BSL-2: Clinical specimens, identifying isolates, processing animal tissues. ABSL-2: Parenteral route of challenge.	Some *Coccidioides* spp. are HHS or USDA **Select Agents**
Histoplasma capsulatum	**BSL-3:** Propagating sporulating cultures of H. capsulatum in the mold form, processing soil or other environmental materials known or likely to contain infectious conidia. BSL-2: Clinical specimens, identifying isolates, animal tissues, mold cultures, identifying cultures in routine diagnostic laboratories ABSL-2	

Parasitic Agents - NONE

Rickettsial Agents

Agent Name	Containment Recommendations	Select Agent
Coxiella burnetii Q fever	**BSL-3:** Inoculation, incubation, harvesting of embryonated eggs or cell cultures, necropsy, manipulation of infected tissues. A**BSL-3** BSL-2: A specific plaque-purified clonal isolate of an avirulent (Phase II) strain (Nine Mile) may be safely handled under BSL2. BSL-2: Nonpropagative laboratory procedures, serological examinations, staining of impression smears.	*C. burnetii* is a HHS **Select Agent**.
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	**BSL-3:** All other manipulations, including necropsy, trituration of tissues, inoculation, incubation, and harvesting of embryonated eggs or cell cultures. **BSL-3:** Animal studies with arthropods naturally or experimentally infected with rickettsial agents of human disease. BSL-2: Nonpropagative laboratory procedures, serological, fluorescent antibody procedures, staining of impression smears. ABSL-2: holding of experimentally infected mammals other than arthropods. 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. Containment of new species on a case-by-case basis.	*R. prowazekii* and *R. rickettsii* are HHS or USDA **Select Agents**

Viral Agents

Agent Name	Containment Recommendations	Select Agent
Hantaviruses	**BSL-3**: Cell-culture virus propagation and purification should be carried out in BSL-3 facility using **BSL-3** practices, containment equipment and procedures. Serum or tissue samples from potentially infected rodents should be handled at BSL-2 using **BSL-3** practices, containment equipment and procedures. ABSL-4: All work involving inoculation of virus-containing samples into rodent species permissive for chronic infection. BSL-2: Sera, BSC 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. ABSL-2: Experimentally infected rodent species known not to excrete the virus.	
Hepatitis B Virus, Hepatitis C Virus (formerly known as nonA nonB Virus), Hepatitis D Virus	**BSL-3** may be indicated for activities with potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials. BSL-2 for all activities utilizing known or potentially infectious body fluids and tissues. ABSL-2: Recommended for activities utilizing naturally or experimentally infected chimpanzees or other NHP.	
Herpesvirus simiae (Cerocopithecine herpesvirus I, herpes B virus)	**BSL-3** practices are recommended for handling materials from which B virus is being cultured using appropriate personal protective equipment; 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. 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.	B virus is a Select Agent requiring registration with HHS and/or USDA for possession, use, storage and/or transfer. See BMBL5 Appendix F for additional information.
Human Herpes Virus	**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 separately in another agent summary statement in this section. BSL-2 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. 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.	
Influenza	**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. BSL-2 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. NON-CONTEMPORARY HUMAN INFLUENZA (H2N2) STRAINS should be handled with increased caution. 1918 INFLUENZA STRAIN 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 A**BSL-3** practices, procedures and facilities. • Large laboratory animals such as NHP should be housed in primary barrier systems in A**BSL-3** facilities. HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) **BSL-3** and A**BSL-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 BMBL5 Appendix D).	Strains of HPAI and 1918 influenza virus are **Select Agents** requiring registration with HHS and/or USDA for possession, use, storage and/or transfer. See BMBL5 Appendix F for additional information.
Lymphocytic Choriomeningitis Virus	**BSL-3:** Aerosol production, production quantities, high concentrations of infectious materials, manipulation of infected transplantable tumors, field isolates and clinical materials from human cases. Strains of LCMV that are shown to be lethal in nonhuman primates should be handled at **BSL-3**. A**BSL-3**: Work with infected hamsters. BSL-2: Body fluids, cell culture passage of laboratory adapted strains. ABSL-2: Studies in adult mice with strains requiring BSL-2 containment.	
Poxviruses	A**BSL-3:** Monkeypox work in experimentally or naturally infected animals. BSL-2 facilities with **BSL-3** practices are advised if other work with monkeypox virus is performed by vaccinated personnel. BSL-2 and ABSL-2 plus vaccination, are recommended for work with most other poxviruses. BSL- 4/ABSL-4: All live variola virus work is to be done only within WHO approved facilities; (one is the HHS in Atlanta, one in Russia).	
Rabies Virus (and related lyssaviruses)	BSL-2 and/or ABSL-2: Infectious materials or animals. **BSL-3:** High potential for droplet or aerosol production, large production quantities or high concentrations of infectious materials.	
Retroviruses, Including Human and Simian Immunodeficiency Viruses (HIV and SIV)	**BSL-3:** Activities involving large-scale volumes or preparation of concentrated HIV or SIV. 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. BSL-2 facilities, using **BSL-3** practices for activities for production of research laboratory- scale quantities of HIV or SIV, manipulation of	

	concentrated virus preparations, and procedures that may produce droplets or aerosols. ABSL-2 is appropriate for NHP and other animals infected with HIV or SIV.	
Severe acute respiratory syndrome (SARS) coronavirus	**BSL-3:** SARS-CoV propagation in cell culture, initial characterization of viral agents recovered in cultures of SARS specimens. A**BSL-3** BSL-2: Pathologic examination, processing of 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.	SARS-associated coronavirus (SARS CoV) is a **Select Agent**

Arboviruses and Related Zoonotic Viruses

Agent Name	Containment Recommendations	Select Agent
West Nile Virus (WNV)	**BSL-3/**A**BSL-3:** Manipulations of WNV cultures, animal, vector studies. Dissection of field collected dead birds for histopathology and culture. BSL-2: human diagnostic specimens, although it is unusual to recover virus from specimens obtained from clinically ill patients. Non-invasive procedures performed on dead birds (such as oropharyngeal or cloacal swabs).	
Eastern equine encephalitis (EEE) virus, Venezuelan equine encephalitis (VEE) virus, Western equine encephalitis (WEE) virus	**BSL-3:** Diagnostic, clinical materials, infectious cultures, infected animals or arthropods. Due to the high risk of aerosol infection, additional personal protective equipment, including respiratory protection, should be considered for non-immune personnel. A**BSL-3:** VEE virus, EEE virus and WEE virus HEPA filtration is required on the exhaust system of laboratory and animal facilities using VEE virus.	VEE virus and EEE virus are **Select Agents**
Rift Valley Fever Virus (RVFV)	**BSL-3:** Processing human or animal material in endemic zones or in non-endemic areas in emergency circumstances; 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, for research conducted in non-endemic areas, the USDA may require full **BSL-3**Ag containment. The USDA may require enhanced A**BSL-3** or A**BSL-3** facilities and practices for working with RVFV in the United States.	RVFV is a **Select Agent** The live-attenuated MP-12 vaccine strain is specifically exempted from the Select Agent rules. BSL-2 containment is recommended for this strain.

Toxin Agents

Agent Name	Containment Recommendations	Select Agent
Botulinum Neurotoxin	**BSL-3**: Aerosol or droplet production, larger quantities. 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. BSL-2: Routine dilutions, titrations or diagnostic studies	Botulinum toxin is a **Select Agent**
Staphylococcal enterotoxins (SE)	**BSL-3:** Aerosol, droplet production, large quantities. BSL-2	SE *A,B,C,D,E subtypes are* **Select Agents**