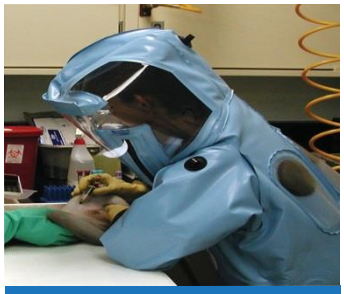

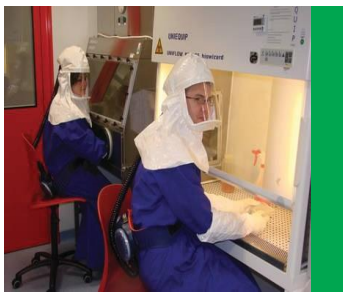

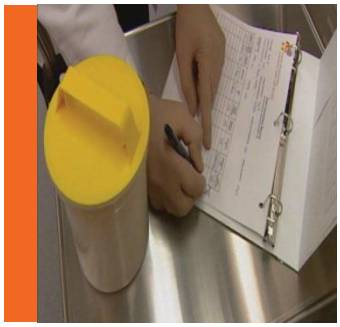

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Select Agents and Toxins Biosafety/ Biocontainment Plan Guidance

(7 CFR Part 331.12, 9 CFR Part 121.12, 42 CFR Part 73.12)

(March 2017)

Centers for Disease Control and Prevention (CDC)
Division of Select Agents and Toxins (DSAT)
Animal and Plant Health Inspection Services (APHIS)
Agriculture Select Agent Services (AgSAS)

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Change/Highlight Section

Revisions: This is a living document subject to ongoing improvement. Feedback or suggestions for improvement from entities registered with the Federal Select Agent Program, as well as the general public, are welcomed. Submit comments directly to the Federal Select Agent Program at:

DSAT: LRSAT@cdc.gov

AgSAS: AgSAS@aphis.usda.gov

Introduction

This document is intended to provide guidance and assist entities in developing and implementing a written biosafety/biocontainment plan, as required by section 12 of the select agent regulations (7 C.F.R. Part 331, 9 C.F.R. Part 121, and 42 C.F.R. Part 73). This template summarizes current regulatory and procedural criteria for registered entities and provides examples for verifying compliance. It does not add to, delete from, or change current regulatory requirements or standards. For entities registered for Tier 1 select agents and toxins that require an occupational health program, reference the Occupational Health Program Guidance for more information. It should be noted that information regarding an occupational health program may be incorporated into the biosafety plan and that two plans are not required.

There are resources available to assist entities in development of biosafety/biocontainment plans such as the "Biosafety in Microbiological and Biomedical Laboratories (BMBL)," "NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)," "Containment Facilities and Safeguards for Exotic Plant Pathogens and Pests" (Robert P. Kahn and S.B. Mathur eds., 1999; copies available upon request at lrsat@cdc.gov or AgSAS@aphis.usda.gov), and "A Practical Guide to Containment: Greenhouse Research with Transgenic Plants and Microbes" (Patricia L. Traynor ed., 2001; copies available upon request at lrsat@cdc.gov or AgSAS@aphis.usda.gov). These resources can be used as guidance to assist in the development of the biosafety/biocontainment plan. However, entities may use other biosafety/biocontainment guidelines and regulations when developing and implementing a written plan. It should be noted that the Federal Select Agent Program inspects registered entities in accordance with these currently nationally recognized standards.

Definitions

As used in this document the following terms have the following meanings:

Decontamination – Disinfection or sterilization of articles contaminated with toxins or agents to make the articles safe for use or disposal.

Disinfection – The elimination of nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects.

Exposure – Any event which results in any person in a registered entity facility or laboratory not being appropriately protected in the presence of an agent or toxin. This may include reasonably anticipated skin,

eye, mucous membrane, or parenteral contact with blood or other potential infectious materials that may result from the performance of a person's duties.

Risk – The potential for an adverse outcome assessed as a function of threats, vulnerabilities, and consequences associated with an incident, event, or occurrence.

Risk Assessment – The process of evaluating the risk(s) arising from a hazard(s), taking into account the adequacy of any existing controls and deciding whether or not the risk(s) is acceptable.

Sterilization – Any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms and viruses. The definition is categorical and absolute (i.e., an item is either sterile or it is not). A sterilization procedure is one that kills all microorganisms, including high numbers of bacterial endospores.

Biosafety/Biocontainment Plan Provision Requirements

Hazardous Characteristics of Select Agents and Toxins

It is important that the biosafety/biocontainment plan contain the hazardous characteristics of each agent or toxin listed on the entity's registration and the biosafety/biocontainment risk associated with laboratory procedures related to the select agent or toxin.

To assist with identifying the hazardous characteristics of each agent or toxin and the biosafety risk associated with laboratory procedures related to the select agent or toxin, the BMBL is an excellent reference and includes agent summary statements that describe the hazards, recommended precautions, additional risks, and levels of containment appropriate for handling select agents and toxins in the laboratory. The BMBL also states that HEPA filtration of exhaust air should be required when working with BSL-4 select agents and toxins as well as:

- Reconstructed 1918 influenza virus
- Rift Valley fever virus
- Venezuelan equine encephalitis virus
- Highly pathogenic avian influenza virus

The NIH Guidelines provides risk assessment, physical containment, and biological containment provisions relating to genetic elements, recombinant nucleic acids and recombinant organisms of select agents and toxins.

In addition, AgSAS has developed Guidelines for Avian Influenza Viruses to assist individuals and entities with developing policies and implementing procedures for working safely with these viruses in the laboratory.

In considering hazardous characteristics of each agent or toxin, the entity should discuss the hazards of agent cross-contamination in laboratories performing work with multiple select agents and agent strains to prevent the accidental transfer of agents. Additional information to minimize the risk of cross-contamination is described in the biosafety and containment considerations in the

Biosafety/Biocontainment Procedures section of this document.

Safeguards for Protecting Against Exposure to Select Agents and Toxins

Section 12(a)(2) of the select agent regulations state that the biosafety/biocontainment plan must include Safeguards in place with associated work practices to protect entity personnel, the public, and the environment from exposure to the select agent or toxin including, but not limited to: personal protective equipment and other safety equipment; containment equipment including, but not limited to, biological safety cabinets, animal caging systems, and centrifuge safety containers; and engineering controls and other facility safeguards..

Personal protective equipment (PPE) and other safety equipment

In determining the PPE and other safety equipment needed, consider the hazardous characteristics of each agent or toxin listed on the entity's registration and the risk associated with laboratory procedures related to the select agent or toxin. The PPE and other safety equipment should focus on:

- Breathing or respiratory protection
- Eye and face protection
- Head protection
- Hearing protection
- Hand/arm protection (gloves, sleeves)
- Foot protection

When considering laboratory clothing, the entity needs to determine what PPE should be worn to prevent hazards from leaving the laboratory (i.e., how clothing can be a fomite to carry BSAT out of laboratories and how the clothing should be cleaned, disinfected, or disposed). Employees should be educated that PPE must not be worn outside the containment laboratory except when transporting samples between labs. It must not be worn (or stored) in break rooms, office areas, toilets, or outside the building. Employees must be properly instructed on how to don (put on) required PPE before entering an area with a potential hazard that requires the use of the PPE. Workers may not remove (doff) required PPE before leaving the area of exposure.

Biological Safety - Personal Protective Equipment (PPE) Requirements*

BSL-1	BSL-2	BSL-3	BSL-4
• **Protective laboratory coats, gowns, or uniforms** recommended to prevent contamination of personal clothing. • **Protective eyewear** worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Personnel who wear contact lenses in laboratories should also wear eye protection.	• **Protective laboratory coats, gowns, smocks, or uniforms** must be worn while working with hazardous materials. • **Eye and face protection** (goggles, mask, face shield or other splatter guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms are handled outside the Biological Safety Cabinet (BSC) or physical containment device.	• **Protective laboratory clothing with a solid-front**, such as tie-back or wrap-around gowns, scrub suits, or coveralls must be worn. • **Eye and face protection** (goggles, mask, face shield or other splash guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials. [All procedures involving the manipulation of infectious materials must be conducted within a	*Use of a positive pressure suit connected to a HEPA filtered airline. The positive pressure suit completely isolates the laboratory worker from the laboratory environment, ensuring there is no contact with potentially hazardous material. Laboratory personnel who work in positive pressure suits require significant training.

• **Gloves** must be worn to protect hands from exposure to hazardous materials.	Personnel who wear contact lenses in laboratories should also wear eye protection. • **Gloves** must be worn to protect hands from exposure to hazardous materials. • **Eye, face and respiratory protection** should be used in rooms containing infected animals.	BSC, or other physical containment devices.] Personnel who wear contact lenses in laboratories must also wear eye protection. • **Gloves** must be worn to protect hands from exposure to hazardous materials. • **Eye, face, and respiratory protection** must be used in rooms containing infected animals.	

Containment equipment

The containment equipment should focus on:

- Primary containment: the first container in direct contact with biohazardous material, as well as other methods to protect personnel and the immediate laboratory environment from exposure to infectious agents. Primary containment requires using proper storage containers, good microbiological technique, and the use of appropriate equipment such as biological safety cabinets. Primary containment may include:
    - Biological safety cabinets
    - Animal/arthropod caging systems
    - Plant growth chambers
    - Centrifuge safety containers
- Secondary containment is the protection of the environment external to the laboratory from exposure to infectious materials and is provided by a combination of facility design and operational practices. Secondary containment may include separation of the laboratory work area from public access, availability of decontamination equipment (e.g., autoclave), and hand washing facilities.

Engineering controls and other facility safeguards

The basic concept behind engineering controls is that, to the extent feasible, the work environment and the biosafety/biocontainment risk associated with the laboratory procedures should be designed to eliminate hazards or reduce exposure to hazards. Engineering controls should be based on the following principles:

- If feasible, design the facility, equipment, or process to remove the hazard.
- If removal is not feasible, enclose the hazard to prevent exposure during normal operations.
- Where complete enclosure is not feasible, establish barriers or local ventilation to reduce exposure to the hazard during normal operations.

The basic types of engineering controls are:

- Process control
- Enclosure and/or isolation of source
- Ventilation

Examples of engineering controls may include:

- Building ventilation/exhaust or HVAC (heating, ventilation and air conditioning) must provide safe, comfortable, breathable environments for all employees and the public, and to minimize exposures to hazardous air contaminants. At BSL-3 and BSL-4, exhaust laboratory air must be directly exhausted to the outside since it is considered potentially contaminated. The exhausted room air can be high-efficiency particulate air (HEPA)-filtered to prevent the hazards from being released to the outside environment. The HVAC exhaust system must be sized to handle both the room exhaust and the exhaust requirements of all containment devices that may be present. Adequate supply air must be provided to ensure appropriate function of the exhaust system.
- Biological safety cabinet (BSC) is an enclosed, ventilated laboratory workspace for safely working with materials contaminated with BSAT. To assist with identifying a BSC, the BMBL is an excellent reference for selecting BSC.
- Effluent Decontamination System (EDS) is a system that sterilizes bio-hazardous liquid waste from bio-containment laboratories or other facilities dealing with potentially dangerous effluents (e.g., pressure cooker). Depending on the work at BSL-3 and all BSL-4 laboratories, Liquid effluents from cabinet room sinks, floor drains, autoclave chambers, and other sources within the cabinet room must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.
- Pathological incinerators, or other approved means, must be provided for the safe disposal of the large carcasses of infected animals. Redundancy and the use of multiple technologies need to be considered and evaluated.
- Anaerobic digesters is a biochemical process in which organic matter is decomposed by bacteria in the absence of oxygen. Digesters must be airtight (no oxygen) for anaerobic digestion to occur.

Risk Management Process Controls

Process control involves the way an activity or process is done to reduce the risk. Monitoring should be done before and after any change is implemented to make sure a change results in lower exposures. Develop biosafety/biocontainment policies that rely on the following principals:

- Process controls should be appropriate for the activities performed and the select agent or toxin in use. Biosafety/biocontainment levels are dependent on the risks of the work being performed.
    - For example, the BMBL recommends BSL-3 practices, containment equipment and facilities for all manipulations of suspect cultures of *Francisella tularensis*. In contrast, BSL-2 practices, containment equipment, and facilities are recommended for diagnostic activities involving infectious cultures of *Bacillus anthracis, Burkholderia mallei, Burkholderia pseudomallei,* and *Yersinia pestis*.
    - For *B. anthracis*, the BMBL recommends BSL-2 practices, containment equipment and facilities for laboratories performing diagnostic activities.
    - For *B. mallei, B. pseudomallei,* and *Y. pestis,* the BMBL recommends BSL-2 practices, containment equipment and facilities are recommended for performing diagnostic activities with all work in a biological safety cabinet.
- Detailed safety measures to ensure that primary and secondary containment are maintained during especially hazardous procedures (e.g., intentional production of select agent infectious aerosols or select toxin aerosols).
- Reference the FSAP Policy Statement: “Laboratory work with the regulated full-length genomes of regulated Risk Group 3 and 4 (RG3 and RG4) agents at one containment level lower than the infectious virus without RNA inactivation.”

- Describe the biosafety and containment procedures employed for experimentally exposed or infected animals or plants, if applicable.
    - When animals or plants are to be infected with or exposed to select agents, describe the administration route(s) employed and the equipment used.
    - Describe in detail appropriate containment of all organic material (select agent-infected carcasses, tissues, plant biomass) until final destruction (e.g., autoclave, incineration, etc.).
    - Describe or reference procedures to monitor animals or plants for accidental infection.
    - Describe procedures to ensure containment of animals accidentally exposed to or infected with select agents. Considerations for developing these procedures include but are not limited to, situations where an airflow reversal has occurred from a room harboring experimentally infected animals to an adjacent room housing native animals; or movement of personnel, equipment, or laboratory waste from a select agent area to a non-select agent area has resulted in accidental exposure.
    - When animals infected with select agents are either loosely housed or housed in open caging, there is an increased potential of room-level select agent contamination. Unless it can be demonstrated that the animal model does not shed the agent, the increased hazard of not using containment caging must be mitigated by procedural or facility enhancements.

Enclosure and Isolation

An enclosure keeps a selected hazard "physically" away from the worker. Enclosed equipment, for example, is tightly sealed and it is typically only opened for moving samples/cultures or for cleaning and maintenance. Examples include "glove boxes" or Class III biosafety cabinets. Care must be taken when the enclosure is opened for maintenance as exposure could occur if adequate precautions are not taken. The enclosure itself must be well maintained to prevent leaks.

Isolation places the hazardous process "geographically" away from the majority of the workers. Common isolation techniques are to create a contaminant-free area either around the equipment or around the employee workstations.

Disinfection, Decontamination or Destruction of Select Agent and Toxin

See the Inactivation guidance for more information on the inactivation of and rendering samples free of select agents and select toxins for future use.

Section 12(a)(3) states that the biosafety/biocontainment plan must contain written procedures for each validated method used for disinfection, decontamination, or destruction, as appropriate, of all contaminated or presumptively contaminated material including, but not limited to:

- Cultures and other materials related to the propagation of select agents or toxins
- Items related to the analysis of select agents and/or toxins
- Personal protective equipment
- Animal caging systems and bedding (if applicable)
- Animal carcasses or extracted tissues and fluids (if applicable)
- Plant biomass (if applicable)

- Laboratory surfaces and equipment
- Surfaces of transport containers
- Effluent material

Development and implementation of written procedures should be based on the following principles:

- Adhere to the concentration and contact time specified by the manufacturer of a disinfectant during laboratory surface decontamination procedures to be effective in decontaminating the select agent and toxin material.
- Ensure that procedures follow any equipment manufacturer guidance on the disinfectants compatible with their equipment.
- Define waste management procedures based on the types of waste generated (e.g., PPE, plates, liquids, eggs, animal caging, carcasses, sharps) and the containers most appropriate for the types of waste being produced.
- Describe in detail safety procedures for decontaminating reusable sharps.
- Describe the procedure for safe transport of waste to the decontamination site, including the location of the decontamination equipment in relation to the laboratory generating the waste. Transport procedures must take into account any safety requirements to protect personnel and the environment during transport.
- Specify the actual method(s) used to decontaminate select agent and toxin waste (e.g., autoclave, incinerators, renderers, tissue digester, chemical, etc.).
- Describe the means of verifying that decontamination equipment is operating correctly, and how often verification is performed (i.e., biological indicators [BIs], confirmation of cycle parameters).
    - For autoclave verification, BIs or parametric monitors should be placed in the center of the load in a manner expected to provide the maximum challenge for steam penetration. When BIs are used, they should be incubated for the length of time stated by the manufacturer and a positive control should be used. The temperature of the material to be autoclaved must be considered when verifying the autoclave parameters (e.g., frozen carcasses will require a longer sterilization time than non-frozen carcasses).
    - For chemical decontamination, the chemical used must be appropriate for the select agent or toxin, and the chemical concentration and contact time must be defined in the procedure. The procedure should also address whether chemicals used for decontamination must be freshly prepared or can be stored, and the shelf life if stored.
- Describe the method(s) used to decontaminate laboratory surfaces and equipment (e.g., chemical surface decontamination, or space fumigation using Vaporized Hydrogen Peroxide, paraformaldehyde, or chlorine dioxide). The method selected must be appropriate for the equipment and the select agents and toxins used in the laboratory. Procedures should indicate contact time required which may be variable depending on agent and equipment.
    - Fumigation used as a means to inactivate select toxins requires the use of a published method or method validation.
    - Fumigation procedures for select agent inactivation should include the use of biological

indicators to verify adequate decontamination.

- Describe how entity personnel are notified of the status of decontamination of laboratory surfaces and equipment.
- Describe how entity personnel are notified of ongoing or completed decontamination activities for laboratory spaces.
- Describe when laboratory surfaces and equipment should be decontaminated.

Handling Select Agents and Toxins in Shared Spaces

Section 12(a)(4) of the regulations requires the entity to describe procedures for the handling of select agents and toxins in the same spaces with non-select agents and toxins in order to prevent unintentional contamination. For example:

- Laboratory work surfaces, equipment, and all select agent and toxin waste that must be decontaminated prior to transitioning to work with non-select agents or toxins.
- How personnel are made aware of the status of any particular room or laboratory at any given time.
- Spatial and/or temporal considerations when performing tissue culture studies.
- Any concurrent work with Reconstructed 1918 Influenza virus and highly pathogenic avian influenza virus.
- Sterilization of all samples at the end of the study/experiment/procedure.

Precautions should be taken to prevent cross-contamination of viral select agents in cell cultures. Some means of preventing accidental transfer of agents between cultures include:

  - Performing all cell culture manipulations in a biosafety cabinet.
  - Working with only one select agent at a time.
  - Decontaminating biosafety cabinet with a surface disinfectant between select agents and toxins.
  - Changing gloves when changing from one select agent to another.
  - Aliquoting growth medium and other reagents so that the same vessel is not used for more than one select agent.

Appendix I: Hazardous Characteristics of Select Agents and Toxins

The content of this chart is intended for instructional use only and does not qualify as an entity specific assessment. The entity must conduct their own assessment when determining the risks and hazardous characteristics associated with the select agent or toxin for which they are registered.

SELECT AGENT OR TOXIN	ENDEMICITY INFORMATION	INFECTIOUS DOSE	LABORATORY SAFETY & CONTAINMENT RECOMMENDATIONS	TREATMENT	DISINFECTANTS
Abrin	Worldwide	LD_{50} of 700 ng/kg IV	BSL-2/ABSL-2	No antidote	0.5% sodium hypochlorite (bleach) for personnel, soak contaminated glassware and equipment in 2.5% bleach + 0.25N NaOH for 8 hours
African horse sickness virus	Endemic regions of Africa	Unknown	BSL-3 with enhancements/ ABSL-3 with enhancements with special consideration to infected vector containment	No treatment	Inactivated by formalin 0.1%/48 hours. Also phenol and iodophores; Inactivated by ether and ß-propiolactone 0.4%.
African swine fever virus (ASFV)	Endemic in most of sub-Saharan Africa including the island of Madagascar and past outbreaks occurred in Europe, South America and the Caribbean.	Unknown	BSL-3 with enhancements/ ABSL-3 with enhancements/ BSL-3-Ag for loosely housed animals with special consideration to infected vector containment	No treatment	Inactivated by 8/1,000 sodium hydroxide (30 min), hypochlorites - 2.3% chlorine (30 min), 3/1,000 formalin (30 min), 3% ortho-phenylphenol (30 min) and iodine compounds. Remains viable for long periods in blood, feces and tissues.
Avian influenza virus	Not endemic to certain region	Unknown	BSL-3/BSL-3-Ag/ABSL-3 with enhancements BSL-3 with enhancements (due to ability of virus to spread by respiratory droplets with potential to cause a pandemic) for HPAIV H5N1 strains with Goose/Guangdong/96-like H5 lineage unless risk assessment by IBC determines otherwise, and mammalian-transmissible by respiratory droplets (additional	Sensitive to the anti-influenza drugs known as neuraminidase inhibitors	Oxidizing agents, sodium dodecyl sulphate, lipid solvents, B-propiolactone,

			requirements for containment, practices and occ. health requirements). Enhancements include use of PAPR, shower out of lab, decontamination of liquid effluents and solid wastes, HEPA filtered exhaust air, sealed ducts, seasonal influenza vaccination and baseline serum banking. http://www.cdc.gov/MMWR/preview/mmwrhtml/rr6206a1.htm	(oseltamivir and zanamivir).	formalin and iodine compounds.
Bacillus anthracis, B. anthracis Pasteur strain, *B. cereus* Biovar *anthracis*	Anthrax in animals is widely endemic in parts of Asia, Africa, Mexico, and Central and South America. Since 1990, animal outbreaks of anthrax in the U.S have occurred in the Midwest, the West and in Texas and Oklahoma.	8,000 to 50,000 organisms by inhalation	BSL-2/ABSL-2 practices for activities involving clinical materials of human or animal origin; BSL-3/ABSL-3 for all manipulations of cultures and for experimental animal studies	Susceptible to penicillin (except for inhalation anthrax in which the mortality remains high); ciprofloxacin, doxycycline, tetracylines, erythromycin, chloramphenicol	Spores are resistant to many disinfectants; susceptible to 2% glutaraldehyde formaldehyde and 5% formalin
Botulinum neurotoxins	Worldwide	Estimated oral or injected toxic dose (serotype A) of 0.001 µg/kg body weight, and an estimated lethal dose by inhalation exposure in humans of approximately 0.07 µg/kg body weight	BSL-2 practices, containment, equipment, and facilities for routine dilutions, titrations, or diagnostic studies BSL-3 practices recommended for aerosol or droplet production and handling of large quantities	Heptavalent Botulinum Antitoxin (HBAT)	Susceptible to many disinfectants - 1% sodium hypochlorite, 70% ethanol; solution of 0.1% sodium hypochlorite or 0.1N NaOH inactivates toxin
Botulinum neurotoxin producing species of *Clostridium*	Worldwide	Cells/spores are not normally toxic for healthy adults	BSL-2/ABSL-2	Susceptible to penicillin, metronidazole, clindamycin, cephalothin, cefoxitin, cefotaxime, chloramphenicol, tetracycline, erythromycin, rifampin, and vancomycin	The vegetative state is susceptible to disinfectants such as 70% ethanol, 0.1% sodium hypochlorite, and 0.1N NaOH. Spores may be resistant to disinfectants.

Brucella abortus, B. melitensis, & B. suis	Worldwide	10-100 organisms	BSL-2/ABSL-2 practices for activities involving clinical materials of human or animal origin; BSL-3/ABSL-3 for all manipulations of cultures and for experimental animal studies	Susceptible to tetracyclines and streptomycin or TMP-SMX; therapy usually consists of a combination of doxycycline and streptomycin	Susceptible to many disinfectants - 1% sodium hypochlorite, 70% ethanol, iodine/alcohol solutions, glutaraldehyde, formaldehyde
Burkholderia mallei	Glanders is endemic in Africa, Asia, the Middle East, and Central and South America.	10-100 organisms	BSL-2/ABSL-2 practices for activities involving clinical materials of human or animal origin; BSL-3/ABSL-3 for all manipulations of cultures and for experimental animal studies	Sensitive to ceftazidime, imipenem, doxycycline, minocycline, ciprofloxacin, gentamicin	Susceptible to many disinfectants; 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde
Burkholderia pseudomallei	Melioidosis is highly endemic in India, Southeast Asia, and Australia, and is found in many tropical regions of the world.	10-100 organisms	BSL-2/ABSL-2 practices for activities involving clinical materials of human or animal origin; BSL-3/ABSL-3 for all manipulations of cultures and for experimental animal studies	TMP-SMX is most effective; susceptible to ceftazidime, imipenem, doxycycline, ciprofloxacinsulphas, chloramphenicol, tetracycline	Susceptible to many disinfectants - 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde
Classical swine fever virus	Endemic in much of Asia, Central and South America, and parts of Europe and Africa	10 $TCID_{50}$	BSL-3 with enhancements. BSL-3-Ag & ABSL-3, both with enhancements with no contact w/ susceptible hosts for 5 days.	No treatment	Inactivated by cresol, sodium hydroxide (2%), formalin (1%), sodium carbonate (4% anhydrous or 10% crystalline, with 0.1% detergent), ionic and non-ionic detergents, strong iodophors (1%) in phosphoric acid.
Conotoxins (Short, paralytic alpha conotoxins)	Worldwide	LD_{50} of 10-100 µg/kg depending upon the species and route of exposure	BSL-2 / ABSL-2	No antidote	2.5% NaOCl or with a combination of 0.25% NaOCl and 0.25N NaO

Coxiella burnetti	Worldwide	10 organisms by inhalation route	BSL-2/ABSL-2 for nonpropagative laboratory procedures, including serological examinations and staining of impression smears BSL-3/ABSL-3 for activities involving the inoculation, incubation, and harvesting of embryonated eggs or tissue cultures, the necropsy of infected animals and the manipulation of infected tissues	Resistant to many antibiotics; tetracycline, chloramphenicol and rifampin may be effective.	Susceptibility to sodium hypochlorite, formalin, phenols varies; susceptible to ethanol, glutaraldehyde and gaseous formaldehyde (humidity control is essential).
Crimean-Congo haemorrhagic fever virus	Eastern Europe, particularly in the former Soviet Union, throughout the Mediterranean, in northwestern China, central Asia, southern Europe, Africa, the Middle East, and the Indian subcontinent	Unknown	BSL-4/ABSL-4	Sensitive to ribavirin	Susceptible to 1% hypochlorite, 2% glutaraldehyde
Diacetoxyscirpenol	Worldwide	LD_{50} of 10 mg/kg IV	BSL-2/ABSL-2	No antidote; ingested (swallowed) toxins are absorbed with a powerful sorbent such as superactivated charcoal	1.0% sodium hypochlorite + 0.1M NaOH for 1 hour contact time
Eastern Equine Encephalitis Virus	Most cases of EEE have been reported from Florida, Georgia, Massachusetts, and New Jersey, and it occurs elsewhere in the U.S. around freshwater hardwood swamps.	A single bite of an infectious mosquito.	BSL-3/ABSL-3	Currently, no treatment is available. Symptomatic treatment is given to maintain vital functions of the body. Passive and active physiotherapy is used during the recovery phase.	Susceptible to disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, 70% ethanol
Ebola Virus	Africa	1 - 10 organisms	BSL-4/ABSL-4	No treatment	Susceptible to 2% sodium hypochlorite, 2% glutaraldehyde, 5% peracetic acid, 1% formalin

Francisella tularensis	Endemic in North America and parts of Europe and Asia.	5 - 10 organisms by the respiratory route; 10^6 - 10^8 organisms by ingestion	BSL-2 for activities with clinical materials BSL-3/ABSL-3 for all manipulations of cultures and for experimental animal studies	Susceptible to aminoglycosides, streptomycin, gentamycin, tobramycin, kanamycin, tetracyclines, and chloramphenicol	Susceptible to many disinfectants - 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde
Foot-and-mouth disease virus	Endemic in parts of Asia, Africa, the Middle East and South America	10 $TCID_{50}$	BSL-4/ABSL-4	No treatment	pH sensitive and virions are inactivated when exposed to pH below 6.5 and above 11. The virus in serum or other organic material will survive drying and can be carried on inanimate objects.
Goat pox virus	Africa and Asia, the Middle East, and most of the Indian subcontinent	$10^{2.7}$ to $10^{4.4}$ $TCID_{50}$ per gram	BSL-3 with enhancements. ABSL-3 with animal facility enhancements.	No treatment	Inactivated by phenol (2%) in 15 min. Sensitive to detergents, e.g., sodium dodecyl sulphate. Can survive for many years in dried scabs at ambient temperatures. Virus remains viable in wool for 2 months and in premises for as long as 6 months.
Hendra virus	Queensland and New South Wales in Australia	Unknown	ABSL4/BSL4	Susceptible to Ribavirin	Inactivated by 0.1% formalin and 0.5% household bleach
Lassa Fever Virus	Endemic in parts of west Africa including Sierra Leone, Liberia, Guinea and Nigeria.	One to 10 aerosolized organisms	BSL-4/ABSL-4	Susceptible to Ribavirin	Susceptible to 0.5 % sodium hypochlorite, phenolic compounds, 3 % acetic acid (pH 2.5), lipid solvents and detergents such as SDS, formaldehyde and

					paraformaldehyde fixation, formaldehyde fumigation, and β-propiolactone
Lujo virus	Africa	One to 10 aerosolized organisms	BSL-4/ABSL-4	No treatment	Inactivated by 0.1% formalin and 0.5% household bleach
Lumpy skin disease virus	Africa and Asia, the Middle East, and most of the Indian subcontinent	$10^{2.7}$ to $10^{4.4}$ $TCID_{50}$ per gram	BSL-4/ABSL-4	No treatment	Susceptible to phenol (2%/15 min). The virus persists in necrotic skin for a minimum of 33 days and remains viable in lesions in air-dried hides for a minimum of 18 days at ambient temperature.
Marburg virus	Sub-Saharan Africa	Unknown; less than 10 infectious units by aerosol for non-human primates	BSL-4/ABSL-4	Susceptible to Ribavirin	Susceptible to 2% sodium hypochlorite, 2% glutaraldehyde, 5% peracetic acid, 1% formalin
Monkeypox virus	Monkeypox outbreaks have been reported in humans in central and western African countries. A 2003 outbreak in the U.S. is the only time human monkeypox is documented outside Africa.	10-100 virions	BSL-3/ABSL-3	Cidofovir	Orthopoxviruses are susceptible to 0.5% sodium hypochlorite, chloroxylenol-based household disinfectants, glutaraldehyde, formaldehyde, and paraformaldehyde.
Mycoplasma capricolum	Africa, Asia, the Middle East, Eastern Europe, and the former Soviet Union	Unknown	BSL-3/ABSL-3	Susceptible to erythromycin, tylosin, tetracycline, or streptomycin	Sodium hypochlorite (bleach)
Mycoplasma mycoides	Widespread in sub-Saharan Africa, including countries in the West, South, East, and Central	1×10^8 organisms subcutaneously and 2×10^9	BSL-3/ABSL-3	No treatment	Inactivated by mercuric

	regions of Africa	organisms intravenously			chloride, phenol, and formaldehyde solution
Newcastle disease virus	Endemic in poultry in most of Asia, Africa, and some countries of North and South America	10_6 EID_{50} per chicken	BSL-2 – low virulence virus or diagnostic accessions. BSL-3/ABSL-3 & BSL-3-Ag with enhancements, including no contact with susceptible species for 5 days.	No treatment	Sensitive to most disinfectants
Nipah virus	Queensland and New South Wales in Australia	Unknown	ABSL4/BSL4	Susceptible to Ribavirin	Inactivated by 0.1% formalin and 0.5% household bleach
Peste des petits ruminants virus	Parts of Africa and Asia, and most of the Middle East	Unknown	BSL-3 with enhancements including no contact with susceptible species for 5 days. ABSL-3 & BSL-3-Ag.	Susceptible to chloramphenicol, penicillin and streptomycin	Inactivated by many disinfectants including alkalis (sodium carbonate, sodium hydroxide), halogens (sodium hypochlorite), phenolic compounds, citric acid, alcohols and iodophores
Ralstonia solanacearum	Tropical, subtropical, and some temperate regions of the world	Natural wounds (created by excision of flowers, genesis of lateral roots) and unnatural ones (by agricultural practices or nematodes and xylem-feeding bugs attack)	BSL-2	No treatment	Alcohol
Rathayibacter toxicus	Australia and South Africa	3-6 mg/kg/ body weight	BSL-2	No treatment	Alcohol
Reconstructed 1918 Influenza virus	Not endemic to certain region	Unknown	BSL-3/ABSL-3 HEPA filtration of laboratory exhaust air	Susceptible to rimantadine (Flumadine) and oseltamivir (Tamiflu).	Susceptible to 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, formalin and iodine compounds.

Ricin	Worldwide	LD_{50}: 2.7 µg/kg	BSL-2 / ABSL-2	No antidote	0.5% sodium hypochlorite (bleach) for personnel, soak contaminated glassware and equipment in 2.5% bleach + 0.25N NaOH for 8 hours
Rickettsia prowazekii	Africa (Ethiopia, Nigeria and Burundi), Mexico, Central America, South America, Eastern Europe, Afghanistan, Northern India, China and the United States	< 10 rickettsial particles	BSL-3/ABSL-3	Susceptible to tetracyclines, chloramphenicol, or doxycycline	1% sodium hypochlorite, 4% formaldehyde, 2% glutaraldehyde, 70% ethanol, 2% peracetic acid, 3- 6% hydrogen peroxide and 0.16% iodine
Rift Valley fever virus	Eastern and southern Africa but also exists in sub-Saharan Africa and Madagascar	A single bite of an infectious mosquito	BSL-3/ABSL-3, HEPA filtration of laboratory exhaust air	Susceptible to Ribavirin	Common disinfectants, solvents and dry heat
Rinderpest virus	South Asia, the Near East and eastern Africa	Unknown	BSL-3/ABSL-3	No treatment	Susceptible to most common disinfectants (phenol, cresol, sodium hydroxide 2%
SARS-associated coronavirus (SARS-CoV)	Since the 2003 outbreak which spread to more than two dozen countries in North and South America, Europe and Asia, there have been no known cases of SARS anywhere in the world.	Unknown	BSL-3/ABSL-3	Corticosteroids and ribavirin.	Inactivated by common disinfection measures such as a 5 minute contact of household bleach, ice-cold acetone, ice-cold acetone/methanol mixture (40:60), 70% ethanol (10 minutes), 100% ethanol (5 minutes) paraformaldehyde, and glutaraldehyde.
Saxitoxin	Worldwide	LD_{50}: 8 µg/kg	BSL-2 / ABSL-2	No antidote	Inactivated by strong alkalis, 0.5% sodium hypochlorite.

Sheep pox virus	Africa and Asia, the Middle East, and most of the Indian subcontinent	$10^{2.7}$ to $10^{4.4}$ $TCID_{50}$ per gram	BSL-3 with enhancements. ABSL-3 with animal facility enhancements.	No treatment	Inactivated by phenol (2%) in 15 min. Sensitive to detergents, e.g., sodium dodecyl sulphate. Can survive for many years in dried scabs at ambient temperatures. Virus remains viable in wool for 2 months and in premises for as long as 6 months.
South American Haemorrhagic Fever viruses (Chapare, Guanarito, Junin, Machupo, Sabia)	South America	1 -10 organisms	BSL-4/ABSL-4	Sensitive to ribavirin	Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde
Staphylococcal enterotoxins A,B,C,D,E subtypes	Worldwide	20 ng/kg - 500 ng/kg	BSL-2 / ABSL-2	No antidote	0.5% sodium hypochlorite
Swine vesicular disease virus	Europe	Unknown	BSL-3 with enhancements including no contact with susceptible species for 5 days. ABSL-3 – with animal facility enhancements.	No treatment	Inactivated by sodium hydroxide
Synchytrium endobioticum	Worldwide	Unknown	BSL-2	No treatment	Alcohol
T-2 toxin	Worldwide	LD_{50} = 4 mg/kg IV	BSL-2 / ABSL-2	No antidote	0.5% sodium hypochlorite
Tetrodotoxin	Worldwide	LD_{50}: 8 µg/kg	BSL-2 / ABSL-2	No antidote	0.5% sodium hypochlorite

Tick-borne encephalitis complex (flavi) viruses: Far Eastern subtype Siberian subtype Kyasanur Forest disease virus Omsk hemorrhagic fever virus	Endemic in focal areas of Europe and Asia	Unknown	BSL-4/ABSL-4	No treatment	1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, and 70% ethanol.
Variola major virus/ Variola minor virus	Eradicated except for laboratory research purposes only	10 to 100 organisms	BSL-4/ABSL-4	No treatment (Smallpox vaccine)	Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, quaternary ammonia.
Venezuelan equine encephalitis virus	Outbreaks have been reported in Latin America and the Everglades.	1 viral unit - subcutaneous	BSL-3/ABSL-3 HEPA filtration of laboratory exhaust air	No specific treatment available. Supportive treatment may be given to alleviate symptoms.	Susceptible to disinfectants - 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, formaldehyde
Xanthomonas oryzae	Worldwide	Unknown	BSL-2	No treatment	Alcohol
Yersinia pestis	Plague epidemics have occurred in Africa, Asia, and South America, but most human cases since the 1990s have occurred in Africa. The western U.S. is also a plague-endemic area.	10-500 organisms	BSL-2/ABSL-2 practices for activities involving clinical materials of human or animal origin; BSL-3/ABSL-3 for all manipulations of cultures and for experimental animal studies	Sensitive to streptomycin, tetracycline, chloramphenicol (for cases of plague meningitis), kanamycin (for neonates).	Susceptible to many disinfectants - 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, iodines, phenolics, formaldehyde

Appendix II: Example Procedural Risks

		Mitigating Factors (A risk assessment for each experiment is necessary, as well as risk communications)						
	Procedural Risks*	PPE	BSC/ Primary Containment	Engineering Controls/ Secondary Containment	Biosafety SOP(s)/ Training	Occ. Health Plan	Gasket on Lid	Notes
	Propogation	X	X	X	X			Sterility testing and use of non-viable/exempt strains
	Lack of Appropriate Immunizations					X		
Aerosol Producing	Vortexing	X	X	X				
	Centrifuging	X	X	X	X		X	Use safety cup (if available)
	Sonicating	X	X	X	X			Use hearing protection
	Pipetting	X	X	X	X			
	Blending	X	X	X	X			
	Homgenizing	X	X	X	X			
	Shakers	X	X	X				
	Lyophilization	X	X	X	X		X	
	Flow Cytometry/ Culture Manipulation	X	X	X	X			
	Automated plating/ Plate washing	X	X	X	X			
	Spills/Splashes/Sprays	X	X	X	X			Use spill kit
	Mouth pipetting and other ingestion forms	X			X	X		
	Cell Line/ Culture manipulation	X	X	X	X			
	Pressure column chromatography	X		X	X			Avoid using glass columns when possible
Animal Work	Injection Procedures	X			X			
	Loosely Housed	X	X	X	X			
	Aerosol Exposure	X	X	X	X			
	Bedding changing and disposal procedures	X	X	X	X			
	Necropsy/ Harvesting tissues	X	X	X	X			
	Animal Bites	X	X	X	X			
	Use of Sharps	X			X			
	Inadequate Training				X			
	Inadequate Safety Equipment				X			
	Inadequate Facilities			X	X			
	Waste Handling and Inactivation Procedures	X		X	X			
	Decontamination	X			X			
	Selection and Use of PPE				X			
	Inadequate Signage/ Labeling				X			

***List is non-comprehensive.**