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Decontamination

Cabinet Surface Decontamination

With the cabinet blower running, all containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the workday, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back and the interior of the glass. If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary. Investigators should remove their gloves and gowns in a manner to prevent contamination of unprotected skin and aerosol generation and wash their hands as the final step in safe microbiological practices. The cabinet blower may be turned off after these operations are completed, or left on.

Small spills within the operating BSC can be handled immediately by removing the contaminated absorbent paper toweling and placing it into the biohazard bag or receptacle. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately cleaned up with a towel dampened with an appropriate decontaminating solution. Gloves should be changed after the work surface is decontaminated and before placing clean absorbent toweling in the cabinet. Hands should be washed whenever gloves are changed or removed.

Spills large enough to result in liquids flowing through the front or rear grilles require decontamination that is more extensive. All items within the cabinet should be surface decontaminated and removed. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.

Twenty to 30 minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. Manufacturer's directions should be followed. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag. The drain pan should be emptied into a collection vessel containing disinfectant. A hose barb and flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. This procedure serves to minimize aerosol generation. The drain pan should be flushed with water and the drain tube removed.

Should the spilled liquid contain radioactive material, a similar procedure can be followed. Radiation safety personnel should be contacted for specific instructions.

Periodic removal of the cabinetwork surface and/or grilles after the completion of drain pan decontamination may be justified because of dirty drain pan surfaces and grilles, which ultimately could occlude the drain valve or block airflow. However, extreme caution should be observed on wiping these surfaces to avoid injury

from broken glass that may be present and sharp metal edges. Always use disposable paper toweling and avoid applying harsh force. Wipe dirty surfaces gently. Never leave toweling on the drain pan because the paper could block the drain valve or the air passages in the cabinet.

Gas Decontamination

BSCs that have been used for work involving infectious materials must be decontaminated before HEPA filters are changed or internal repair work is done.[20-23] Before a BSC is relocated, a risk assessment considering the agents manipulated within the BSC must be performed to determine the need and method for decontamination. The most common decontamination method uses formaldehyde gas, although more recently, hydrogen peroxide vapor[21] and chlorine dioxide gas have been used successfully.

Section VI—Facility and Engineering Requirements

Secondary Barriers

Whereas BSCs are considered to be the primary containment barrier for manipulation of infectious materials, the laboratory room itself is considered to be the secondary containment barrier.[24] Inward directional airflow is established[25] by exhausting a greater volume of air than is supplied to a given laboratory and by drawing makeup air from the adjacent space. This is optional at BSL-2 but must be maintained at BSL-3 and BSL-4.[1,26] The air balance for the entire facility should be established and maintained to ensure that airflow is from areas of least to greater potential contamination.

Building Exhaust

At BSL-3 and BSL-4, exhaust laboratory air must be directly exhausted to the outside since it is considered potentially contaminated. This concept is referred to as a dedicated, single-pass exhaust system. The exhausted room air can be HEPA-filtered when a high level of aerosol containment is needed, which is always true at BSL-4 and may be optional at BSL-3. When the building exhaust system is used to vent a ducted BSC, the system must have sufficient capacity to maintain the exhaust flow if changes in the static pressure within the system should occur. The connection to a BSC must be constant air volume (CAV).

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. Right angle bends changing duct diameters and transitional connections within the systems will add to the demand on the exhaust fan. The building exhaust air should be discharged away from supply air intakes, to prevent re-entrainment of laboratory exhaust air into the building air supply system. Refer to recognized design guides for locating the exhaust terminus relative to nearby air intakes.[27]

Utility Services

Utility services needed within a BSC must be planned carefully. Protection of vacuum systems must be addressed (Figure 12). Electrical outlets inside the cabinet must be protected by ground fault circuit interrupters and should be supplied by an independent circuit. When propane or natural gas is provided, a clearly marked emergency gas shut-off valve outside the cabinet must be installed for fire safety. All non-electrical utility services should have exposed, accessible shut-off valves. The use of compressed air within a BSC must be carefully considered and controlled to prevent aerosol production and reduce the potential for vessel pressurization.

Ultraviolet Lamps

Ultraviolet (UV) lamps are not recommended in BSCs[8] nor are they necessary. If installed, UV lamps must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light. The lamps should be checked weekly with a UV meter to ensure that the appropriate intensity of UV light is being emitted. UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer. If the cabinet has a sliding sash, close the sash when operating the UV lamp.

BSC Placement

BSCs were developed (see Section I) as workstations to provide personnel, environmental and product protection during the manipulation of infectious microorganisms. Certain considerations must be met to ensure maximum effectiveness of these primary barriers. Whenever possible, adequate clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance and to ensure that the cabinet air re-circulated to the laboratory is not hindered. A 12 to 14 inch clearance above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter surface[28,29] and for exhaust filter changes. When the BSC is hard-ducted or connected by a canopy unit to the ventilation system, adequate space must be provided so that the configuration of the ductwork will not interfere with airflow. The canopy unit must provide adequate access to the exhaust HEPA filter for testing.

The ideal location for the biological safety cabinet is remote from the entry (i.e., the rear of the laboratory away from traffic), since people walking parallel to the face of a BSC can disrupt the air curtain.[16,20,30] The air curtain created at the front of the cabinet is quite fragile, amounting to a nominal inward and downward velocity of 1 mph. Open windows, air supply registers, portable fans or laboratory equipment that creates air movement (e.g., centrifuges, vacuum pumps) should not be located near the BSC. Similarly, chemical fume hoods must not be located close to BSCs.

HEPA Filters

HEPA filters, whether part of a building exhaust system or part of a cabinet, will require replacement when they become loaded to the extent that sufficient airflow can no longer be maintained. In most instances, filters must be decontaminated before removal. To contain the formaldehyde gas typically used for microbiological decontamination, exhaust systems containing HEPA filters require airtight dampers to be installed on both the inlet and discharge side of the filter housing. This ensures containment of the gas inside the filter housing during decontamination. Access panel ports in the filter housing also allow for performance testing of the HEPA filter. (See Section VII.)

A bag-in/bag-out filter assembly[3,17] (Figure 13) can be used in situations where HEPA filtration is necessary for operations involving biohazardous materials and hazardous or toxic chemicals. The bag-in/bag-out system is used when it is not possible to gas or vapor decontaminate the HEPA filters, or when hazardous chemicals or radionuclides have been used in the BSC, and provides protection against exposure for the maintenance personnel and the environment. Note, however, that this requirement must be identified at the time of purchase and installation; a bag-in/bag-out assembly cannot be added to a cabinet after-the-fact without an extensive engineering evaluation.

Section VII — Certification of BSCs

Development of Containment Standards

The evolution of containment equipment for varied research and diagnostic applications created the need for consistency in construction and performance. Federal Standard 209[a,32,33] was developed to establish classes of air cleanliness and methods for monitoring clean workstations and clean rooms where HEPA filters are used to control airborne particulates.

The first "standard" to be developed specifically for BSCs[12] served as a Federal procurement specification for the NIH Class II, Type 1 (now called Type A1) BSC, which had a fixed or hinged front window or a vertical sliding sash, vertical downward airflow and HEPA-filtered supply and exhaust air. This specification described design criteria and defined prototype tests for microbiological aerosol challenge, velocity profiles, and leak testing of the HEPA filters. A similar procurement specification was generated[31] when the Class II, Type 2 (now called Type B1) BSC was developed.

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a Federal Standard No. 209E9 has been replaced by ISO 14644. This standard does not apply to BSCs and should not be considered a basis for their performance or integrity certification. However, the methodology of ISO 14644 can be used to quantify the particle count within the work area of a BSC. ISO 14644 defines how to classify a clean room/clean zone. Performance tests and procedures needed to achieve a specified cleanliness classification are outlined by the Institute of Environmental Sciences and Technology's IEST-RP-CC-006.

National Sanitation Foundation (NSF) Standard #49 for Class II BSCs was first published in 1976, providing the first independent standard for design, manufacture and testing of BSCs. This standard "replaced" the NIH specifications, which were being used by other institutions and organizations purchasing BSCs. NSF/ANSI Standard 49—2007[b,8] incorporates current specifications regarding design, materials, construction, and testing. This Standard for BSCs establishes performance criteria and provides the minimum testing requirements that are accepted in the United States. Cabinets, which meet the Standard and are certified by NSF bear an NSF Mark.

NSF/ANSI Standard 49—2007 pertains to all models of Class II cabinets (Type A1, A2, B1, B2) and provides a series of specifications regarding:

- Design/construction
- Performance
- Installation recommendations
- Recommended microbiological decontamination procedure
- References and specifications pertinent to Class II Biosafety Cabinetry

Annex F of NSF/ANSI Standard 49—2007, which covers field-testing of BSCs, is now a normative part of the Standard. This Standard is reviewed periodically by a committee of experts to ensure that it remains consistent with developing technologies.

The operational integrity of a BSC must be validated before it is placed into service and after it has been repaired or relocated. Relocation may break the HEPA filter seals or otherwise damage the filters or the cabinet. Each BSC should be tested and certified at least annually to ensure continued, proper operation.

On-site field-testing (NSF/ANSI Standard 49—2007 Annex F plus Addendum #1) must be performed by experienced, qualified personnel. Some basic information is included in the Standard to assist in understanding the frequency and kinds of tests to be performed. In 1993, NSF began a program for accreditation of certifiers based on written and practical examinations. Education and training programs for persons seeking accreditation as qualified to perform all field certification tests are offered by a variety of organizations. Selecting competent individuals to perform testing and certification is important. It is suggested that the institutional BSO be consulted when identifying companies qualified to conduct the necessary field performance tests.

b The standard can be ordered from the NSF for a nominal fee at NSF International, 789 North Dixboro Road, P.O. Box 130140, Ann Arbor, Michigan, 48113-0140; Telephone: 734-769-8010; Fax: 734-769-0190; e-mail: info@nsf.org; Telex 753215 NSF INTL.

It is strongly recommended that, whenever possible, accredited field certifiers are used to test and certify BSCs. If in-house personnel are performing the certifications, then these individuals should become accredited.

The annual tests applicable to each of the three classes of BSCs are listed in Table 3. Table 4 indicates where to find information regarding the conduct of selected tests. BSCs consistently perform well when proper annual certification procedures are followed; cabinet or filter failures tend to occur infrequently.

Performance Testing BSCs in the Field

Class II BSCs are the primary containment devices that protect the worker, product and environment from exposure to microbiological agents. BSC operation, as specified by NSF/ANSI Standard 49—2007, Annex F plus Addendum #1 needs to be verified at the time of installation and, as a minimum, annually thereafter. The purpose and acceptance level of the operational tests (Table 3) ensure the balance of inflow and exhaust air, the distribution of air onto the work surface, and the integrity of the cabinet and the filters. Other tests check electrical and physical features of the BSC.

A. *Down flow Velocity Profile Test:* This test is performed to measure the velocity of air moving through the cabinet workspace, and is to be performed on all Class II BSCs.

B. *Inflow Velocity Test:* This test is performed to determine the calculated or directly measured velocity through the work access opening, to verify the nominal set point average inflow velocity and to calculate the exhaust airflow volume rate.

C. *Airflow Smoke Patterns Test:* This test is performed to determine if: 1) the airflow along the entire perimeter of the work access opening is inward; 2) if airflow within the work area is downward with no dead spots or refluxing; 3) if ambient air passes onto or over the work surface; and 4) if there is no escape to the outside of the cabinet at the sides and top of the window. The smoke test is an indicator of airflow direction, not velocity.

D. *HEPA Filter Leak Test:* This test is performed to determine the integrity of supply and exhaust HEPA filters, filter housing and filter mounting frames while the cabinet is operated at the nominal set point velocities. An aerosol in the form of generated particulates of dioctylphthalate (DOP) or an accepted alternative (e.g., poly alpha olefin (PAO), di(2-ethylhexyl) sebecate, polyethylene glycol and medical grade light mineral oil) is required for leak-testing HEPA filters and their seals. The aerosol is generated on the intake side of the filter and particles passing through the filter or around the seal are measured with a photometer on the discharge side. This test is suitable for ascertaining the integrity of all HEPA filters.

E *Cabinet Integrity Test (A1 Cabinets only):* This pressure holding test is performed to determine if exterior surfaces of all plenums, welds, gaskets and plenum penetrations or seals are free of leaks. In the field, it need only be performed on Type A1 cabinets at the time of initial installation when the BSC is in a free-standing position (all four sides are easily accessible) in the room in which it will be used, after a cabinet has been relocated to a new location, and again after removal of access panels to plenums for repairs or a filter change. This test may also be performed on fully installed cabinets. Cabinet integrity can also be checked using the bubble test; liquid soap can be spread along welds, gaskets and penetrations to visualize air leaks that may occur.

F. *Electrical Leakage and Ground Circuit Resistance and Polarity Tests:* Electrical testing has been taken out of NSF/ANSI 49 Standard—2007 for new cabinets certified under the this Standard. This responsibility has been turned over to UL. All new cabinets must meet UL 61010A-1 in order to be certified by NSF. These safety tests are performed to determine if a potential shock hazard exists by measuring the electrical leakage, polarity, ground fault interrupter function and ground circuit resistance to the cabinet connection. An electrical technician other than the field certification personnel may perform the tests at the same time the other field certification tests are conducted. The polarity of electrical outlets is checked (Table 3, E). The ground fault circuit interrupter should trip when approximately five milliamperes (mA) is applied.

G. *Lighting Intensity Test:* This test is performed to measure the light intensity on the work surface of the cabinet as an aid in minimizing cabinet operator fatigue.

H. *Vibration Test:* This test is performed to determine the amount of vibration in an operating cabinet as a guide to satisfactory mechanical performance, as an aid in minimizing cabinet operator fatigue and to prevent damage to delicate tissue culture specimens.

I. *Noise Level Test:* This test is performed to measure the noise levels produced by the cabinets, as a guide to satisfactory mechanical performance and an aid in minimizing cabinet operator fatigue.

J. *UV Lamp Test:* A few BSCs have UV lamps. When used, they must be tested periodically to ensure that their energy output is sufficient to kill microorganisms. The surface on the bulb should be cleaned with 70% ethanol prior to performing this test. Five minutes after the lamp has been turned on, the sensor of the UV meter is placed in the center of the work surface. The radiation output should not be less than 40 microwatts per square centimeter at a wavelength of 254 nanometers (nm).

Finally, accurate test results can only be assured when the testing equipment is properly maintained and calibrated. It is appropriate to request the calibration information for the test equipment being used by the certifier.

Table 1. Selection of a Safety Cabinet through Risk Assessment

Biological Risk Assessed	Protection Provided			BSC Class
	Personnel	Product	Environmental	
BSL 1 – 3	Yes	No	Yes	I
BSL 1 – 3	Yes	Yes	Yes	II (A1, A2, B1, B2)
BSL – 4	Yes	Yes	Yes	III; II—When used in suit room with suit

Table 2. Comparison of Biosafety Cabinet Characteristics

BSC Class	Face Velocity	Airflow Pattern	Applications	
			Nonvolatile Toxic Chemicals and Radionuclides	Volatile Toxic Chemicals and Radionuclides
I	75	In at front through HEPA to the outside or into the room through HEPA (Figure 2)	Yes	When exhausted outdoors[1,2]
II, A1	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to outside through a canopy unit (Figure 3)	Yes (minute amounts)	No
II, B1	100	30% recirculated, 70% exhausted. Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter (Figures 5A, 5B)	Yes	Yes (minute amounts)[1,2]
I, B2	100	No recirculation; total exhaust to the outside through a HEPA filter (Figure 6)	Yes	Yes (small amounts)[1,2]
II, A2	100	Similar to II, A1, but has 100 lfm intake air velocity and plenums are under negative pressure to room; exhaust air can be ducted to the outside through a canopy unit (Figure 7)	Yes	When exhausted outdoors (FORMALLY "B3") (minute amounts)[1,2]

BSC Class	Face Velocity	Airflow Pattern	Applications	
			Nonvolatile Toxic Chemicals and Radionuclides	Volatile Toxic Chemicals and Radionuclides
III	N/A	Supply air is HELP filtered. Exhaust air passes through two HEPA filters in series and is exhausted to the outside via a hard connection (Figure 8)	Yes	Yes (small amounts)[1,2]

[1] Installation requires a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I or Class II, Type A2 cabinet into a room should not occur if volatile chemicals are used.

[2] In no instance should the chemical concentration approach the lower explosion limits of the compounds.

Table 3. Field Performance Tests Applied to the Three Classes of Biological Safety Cabinets

Test Performed for	Biosafety Cabinet		
	Class I	Class II	Class III
Primary Containment			
Cabinet Integrity	N/A	A (A1 Only)	A
HEPA Filter Leak	Required	Required	Required
Down flow Velocity	N/A	Required	N/A
Face Velocity	Required	Required	N/A
Negative Pressure / Ventilation Rate	B	N/A	Required
Airflow Smoke Patterns	Required	Required	E, F
Alarms and Interlocks	C, D	C, D	Required
Electrical Safety			
Electrical Leakage, etc.	E, D	E, D	E, D
Ground Fault Interrupter	D	D	D

Test Performed for	Biosafety Cabinet Class I	Class II	Class III
Other			
Lighting Intensity	E	E	E
UV Intensity	C, E	C, E	C, E
Noise Level	E	E	E
Vibration	E	E	E

Required Required during certification.
A Required for proper certification if the cabinet is new, has been moved or panels have been removed for maintenance.
B If used with gloves.
C If present.
D Encouraged for electrical safety.
E Optional, at the discretion of the user.
F Used to determine air distribution within cabinet for clean to dirty procedures.
N/A Not applicable.

Table 4. Reference for Applicable Containment Test

Test	Biosafety Cabinet Type Class I	Class II	Class III
HEPA Filter Leak	(F.5)[1]	(F.5)	(F.5)
Airflow Smoke Pattern	No smoke shall reflux out of BSC once drawn in	(F.4)	N/A
Cabinet Integrity	N/A	(F.6)	[p.138 – 141][2]
Face Velocity Open Front	[75-125 lfm]	75 lfm—type A1; 100 lfm type A2, B1 & B2: (F.3)	N/A
Face VelocityGloves Ports / No Gloves	150 lfm	N/A	N/A
Water Gauge Pressure Glove Ports and Gloves	N/A	N/A	(-0.5 “w.c.”) [p. 145]
Down flow Velocity	N/A	(F.2)	N/A

[1] Parenthetical references are to the NSF/ANSI Standard 49—2007; letters and numerals indicate specific sections and subsections.
[2] Bracketed reference [ ] is to the Laboratory Safety Monograph; page numbers are indicated.

Figure 1. HEPA filters are typically constructed of paper-thin sheets of borosilicate medium, pleated to increase surface area, and affixed to a frame. Aluminum separators are often added for stability.

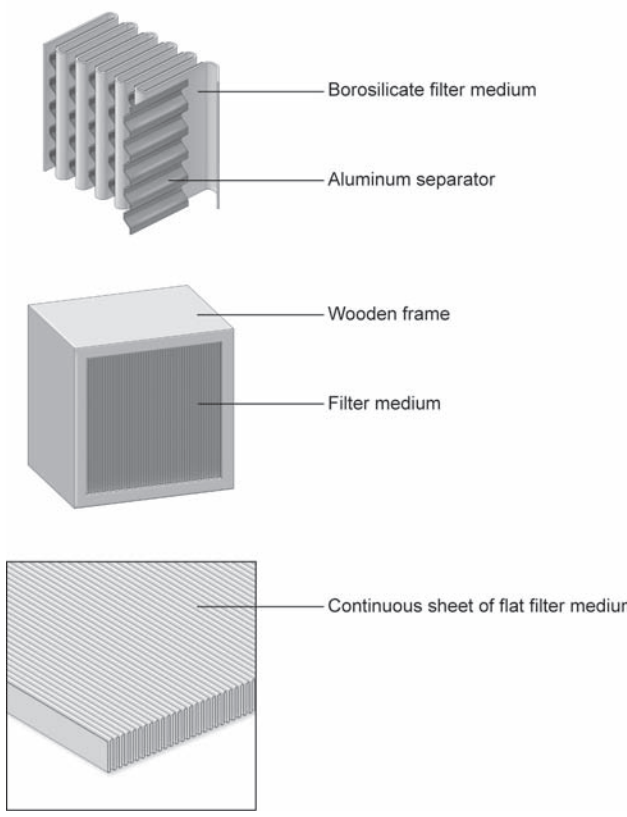


Figure 2. ***The Class I BSC*** (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) exhaust plenum. *Note:* The cabinet needs to be hard connected to the building exhaust system if toxic vapors are to be used.

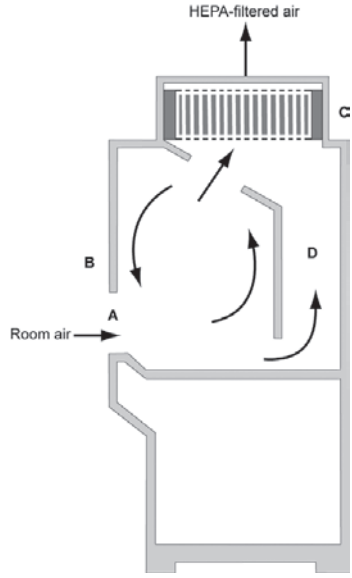


Figure 3. ***The Class II, Type A1 BSC*** (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) common plenum; (F) blower.

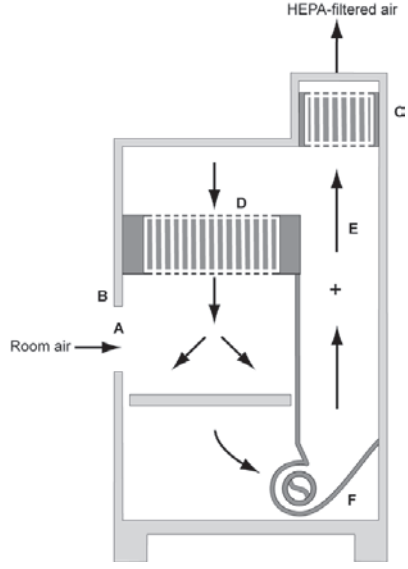


Figure 4. ***Canopy (thimble) unit for ducting a Class II, Type A BSC*** (A) balancing damper; (B) flexible connector to exhaust system; (C) cabinet exhaust HEPA filter housing; (D) canopy unit; (E) BSC. *Note:* There is a 1" gap between the canopy unit (D) and the exhaust filter housing (C), through which room air is exhausted.

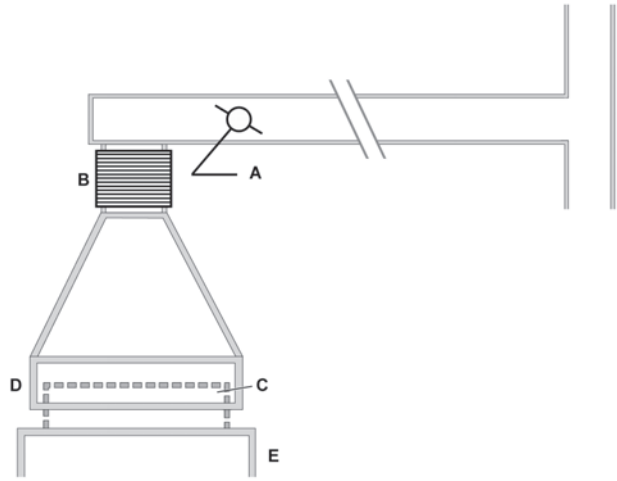


Figure 5A. ***The Class II, Type B1 BSC (classic design)*** (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure dedicated exhaust plenum; (F) blower; (G) additional HEPA filter for supply air. *Note:* The cabinet exhaust needs to be hard connected to the building exhaust system.

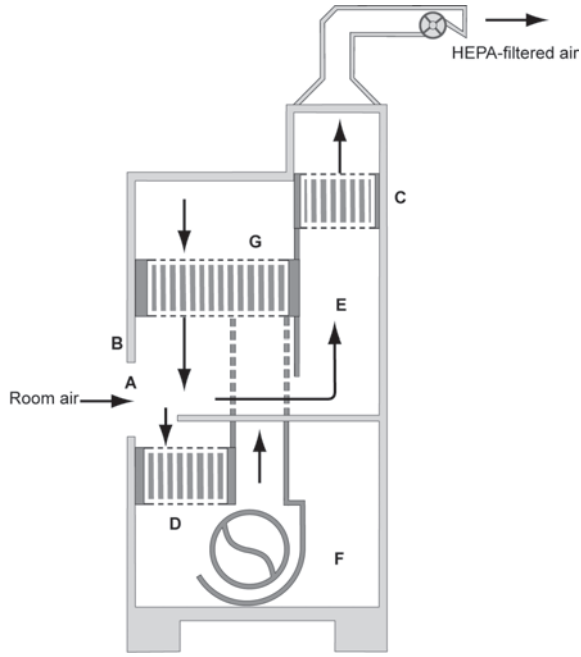


Figure 5B. ***The Class II, Type B1 BSC (bench top design)*** (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply plenum; (E) supply HEPA filter; (F) blower; (G) negative pressure exhaust plenum. *Note:* The cabinet exhaust needs to be hard connected to the building exhaust system.

Connection to the building exhaust system is required.

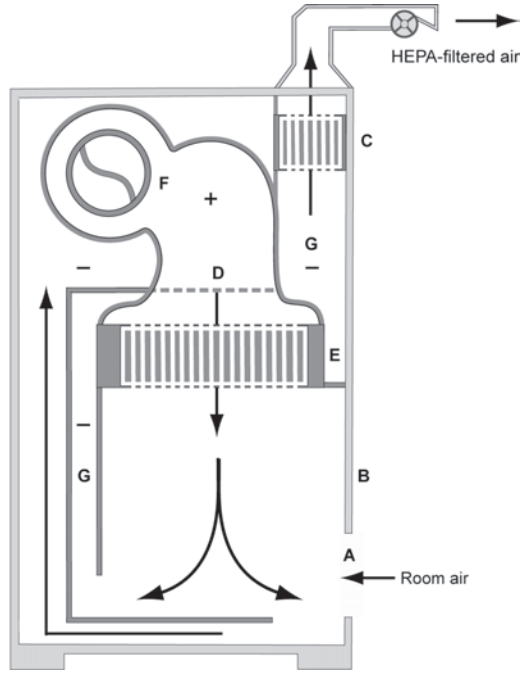


Figure 6. ***The Class II, Type B2 BSC*** (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) negative pressure exhaust plenum. *Note:* The carbon filter in the exhaust system is not shown. The cabinet needs to be hard connected to the building exhaust system.

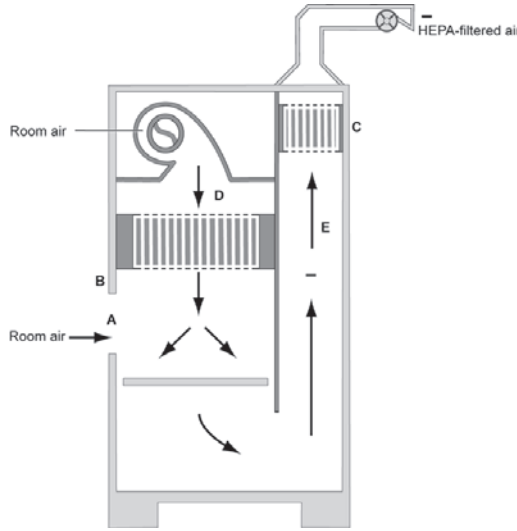


Figure 7. ***The tabletop model of a Class II, Type A2 BSC*** (A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) positive pressure common plenum; (F) negative pressure plenum. The Class II Type A2 BSC **is not equivalent to what was formerly called a *Class II Type B3* unless it is connected to the laboratory exhaust system.** *Note:* The A2 BSC should be canopy connected to the exhaust system.

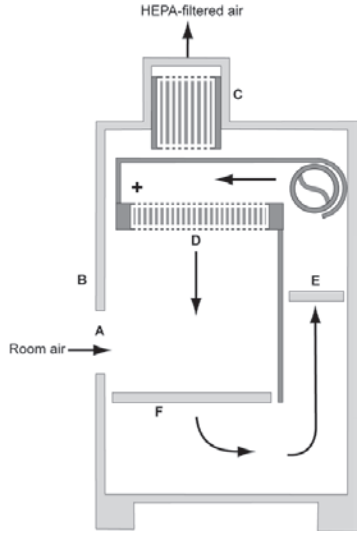


Figure 8. ***The Class III BSC*** (A) glove ports with O-ring for attaching arm-length gloves to cabinet; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) double-ended autoclave or pass-through box. *Note:* A chemical dunk tank may be installed which would be located beneath the work surface of the BSC with access from above. The cabinet exhaust needs to be hard connected to an exhaust system where the fan is generally separate from the exhaust fans of the facility ventilation system. The exhaust air must be double HEPA-filtered or HEPA-filtered and incinerated.

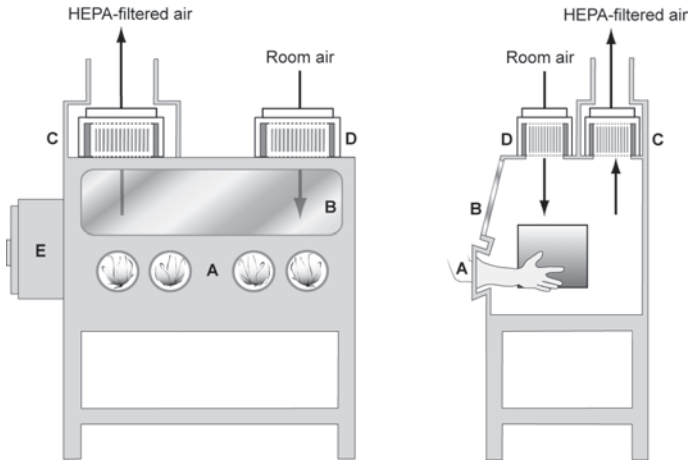


Figure 9A. ***The horizontal laminar flow "clean bench"*** (A) front opening; (B) supply grille; (C) supply HEPA filter; (D) supply plenum; (E) blower.

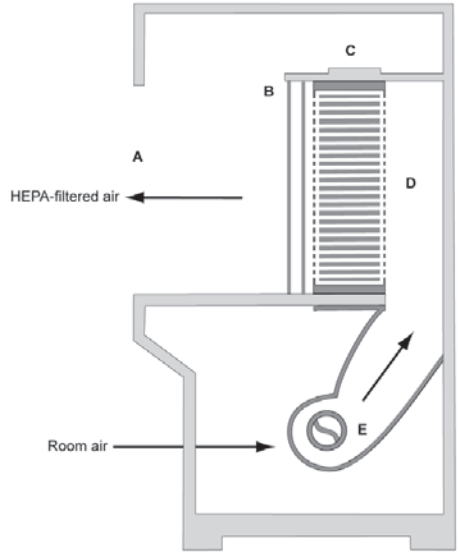


Figure 9B. ***The vertical laminar flow "clean bench"*** (A) front opening; (B) sash; (C) supply HEPA filter; (D) blower. *Note:* Some vertical flow clean benches have recirculated air through front and/or rear perforated grilles.

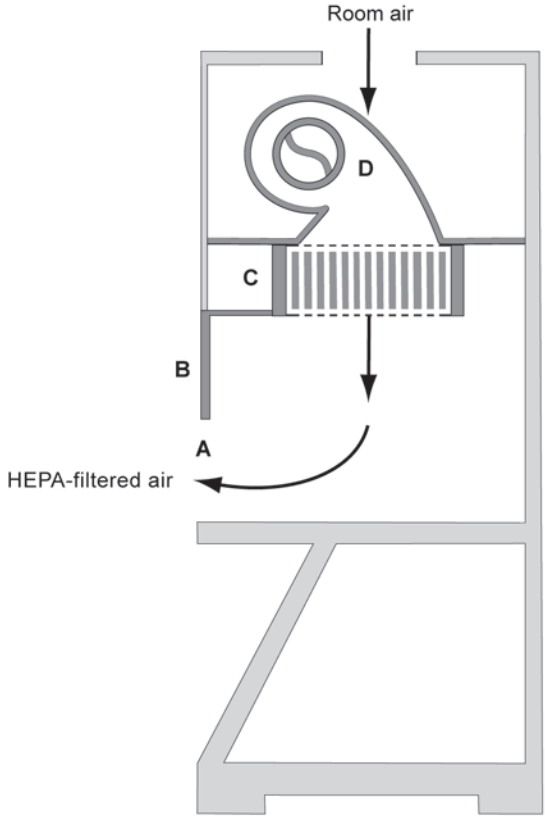


Figure 10. A modified containment cabinet or Class I BSC can be used for labeling infectious microorganisms with I^{125}. (A) arm holes; (B) Lexan^{R} hinged doors; (C) exhaust charcoal filter; (D) exhaust HEPA filter; (E) filter housing with required connection to building exhaust (see also Figure 13).

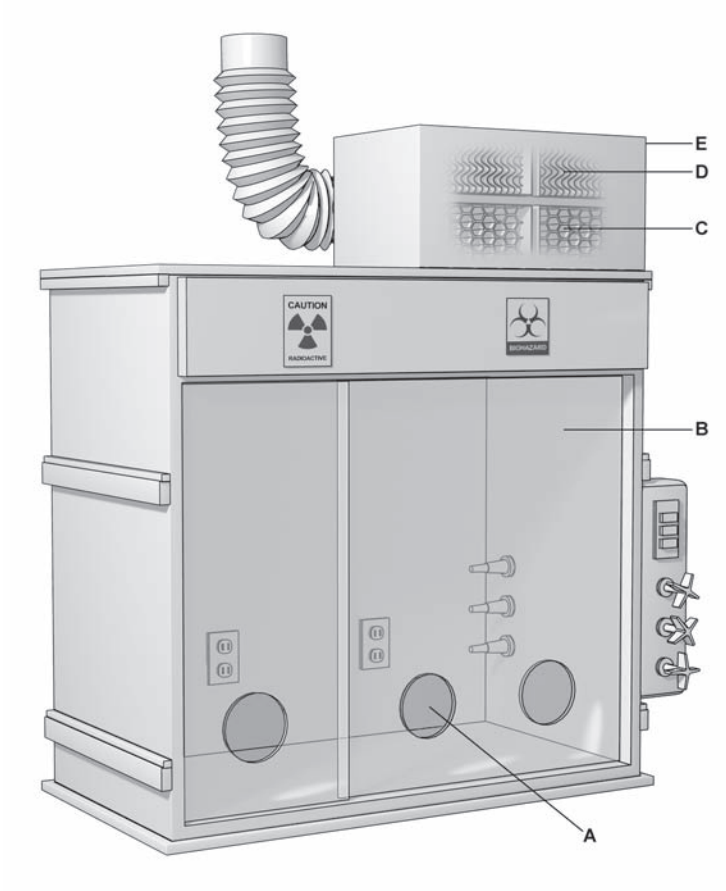


Figure 11. A typical layout for working “clean to dirty” within a Class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right). This arrangement is reversed for left-handed persons.

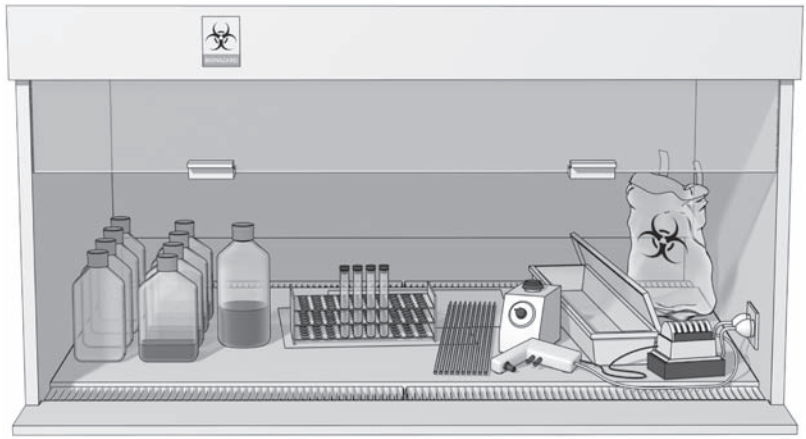


Figure 12. One method to protect a house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

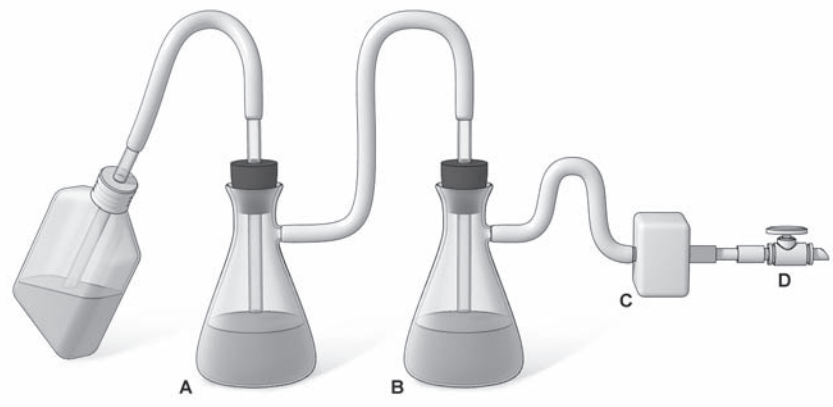
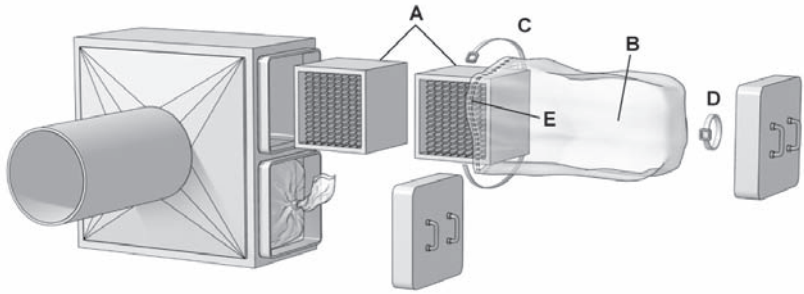


Figure 13. A bag-in-bag-out filter enclosure allows for the removal of the contaminated filter without worker exposure. (A) filters; (B) bags; (C) safety straps; (D) cinching straps; (E) shock cord located in the mouth of the PVC bag restricts the bag around the second rib of the housing lip.



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Appendix B—Decontamination and Disinfection

This section describes basic strategies for decontaminating surfaces, items, and areas in laboratories to eliminate the possibility of transmission of infectious agents to laboratory workers, the general public, and the environment. Factors necessary for environmentally mediated infection transmission are reviewed as well as methods for sterilization and disinfection and the levels of antimicrobial activity associated with liquid chemical germicides. General approaches are emphasized, not detailed protocols and methods. The principles of sterilization and disinfection are stated and compared.

Environmentally Mediated Infection Transmission

Environmentally associated laboratory infections can be transmitted directly or indirectly from environmental sources (e.g., air, contaminated fomites and laboratory instruments, and aerosols) to laboratory staff. Fortunately, LAI are rare events[1] because there are a number of requirements necessary for environmental transmission to occur.[2] Commonly referred to as the "chain of infection" they include: presence of a pathogen of sufficient virulence, relatively high concentration of the pathogen (i.e., infectious dose), and a mechanism of transmission of the pathogen from environment to the host, a correct portal of entry to a susceptible host.

To accomplish successful transmission from an environmental source, all of these requirements for the "chain of infection" must be present. The absence of any one element will prevent transmission. Additionally, the pathogen in question must overcome environmental stresses to retain viability, virulence, and the capability to initiate infection in the host. In the laboratory setting, high concentrations of pathogens can be common. Reduction of environmental microbial contamination by conventional cleaning procedures is often enough to prevent environmentally mediated transmission. However, it is the general practice in laboratories to use sterilization methods to remove the potential for infection transmission.

Principles of Sterilization and Disinfection

In order to implement a laboratory biosafety program it is important to understand the principles of decontamination, cleaning, sterilization, and disinfection. We review here the definitions of sterilization, disinfection, antisepsis, decontamination, and sanitization to avoid misuse and confusion. The definitions and implied capabilities of each inactivation procedure are discussed with an emphasis on achievement and in some cases, monitoring of each state.

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. Sterilization can be accomplished by heat, ethylene oxide gas, hydrogen peroxide gas, plasma, ozone, and radiation (in industry). From an operational standpoint, a sterilization procedure cannot be categorically defined. Rather, the procedure is defined as a process, after which the probability of a microorganism surviving on an item subjected to treatment is less than one in one million (10-6). This is referred to as the "sterility assurance level." [3,4]

Disinfection

Disinfection is generally a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. Disinfection does not ensure an "overkill" and therefore lacks the margin of safety achieved by sterilization procedures. The effectiveness of a disinfection procedure is controlled significantly by a number of factors, each one of which may have a pronounced effect on the end result. Among these are:

- the nature and number of contaminating microorganisms (especially the presence of bacterial spores);
- the amount of organic matter present (e.g., soil, feces, and blood);
- the type and condition of instruments, devices, and materials to be disinfected;
- the temperature.

Disinfection is a procedure that reduces the level of microbial contamination, but there is a broad range of activity that extends from sterility at one extreme to a minimal reduction in the number of microbial contaminants at the other. By definition, chemical disinfection and in particular, high-level disinfection differs from chemical sterilization by its lack of sporicidal power. This is an over simplification of the actual situation because a few chemical germicides used as disinfectants do, in fact, kill large numbers of spores even though high concentrations and several hours of exposure may be required. Non-sporicidal disinfectants may differ in their capacity to accomplish disinfection or decontamination. Some germicides rapidly kill only the ordinary vegetative forms of bacteria such as staphylococci and streptococci, some forms of fungi, and lipid-containing viruses, whereas others are effective against such relatively resistant organisms as *Mycobacterium tuberculosis* var. *bovis*, non-lipid viruses, and most forms of fungi.

Spaulding Classification

In 1972, Dr. Earl Spaulding[5] proposed a system for classifying liquid chemical germicides and inanimate surfaces that has been used subsequently by CDC, FDA, and opinion leaders in the United States. This system, as it applies to device surfaces, is divided into three general categories based on the theoretical risk of infection if the surfaces are contaminated at time of use. From the laboratory perspective, these categories are:

- critical—instruments or devices that are exposed to normally sterile areas of the body require sterilization;
- semi-critical—instruments or devices that touch mucous membranes may be either sterilized or disinfected;
- non-critical—instruments or devices that touch skin or come into contact with persons only indirectly can be either cleaned and then disinfected with an intermediate-level disinfectant, sanitized with a low-level disinfectant, or simply cleaned with soap and water.

In 1991, microbiologists at CDC proposed an additional category, environmental surfaces (e.g., floors, walls, and other "housekeeping surfaces") that do not make direct contact with a person's skin.[6] Spaulding also classified chemical germicides by activity level:

High-level Disinfection

This procedure kills vegetative microorganisms and inactivates viruses, but not necessarily high numbers of bacterial spores. Such disinfectants are capable of sterilization when the contact time is relatively long (e.g., 6 to 10 hours). As high-level disinfectants, they are used for relatively short periods of time (e.g., 10 to 30 minutes). These chemical germicides are potent sporicides and, in the United States, are classified by the FDA as sterilant/disinfectants. They are formulated for use on medical devices, but not on environmental surfaces such as laboratory benches or floors.[7]

Intermediate-level Disinfection

This procedure kills vegetative microorganisms, including *Mycobacterium tuberculosis*, all fungi, and inactivates most viruses. Chemical germicides used in this procedure often correspond to Environmental Protection Agency (EPA)-approved "hospital disinfectants" that are also "tuberculocidal." They are used commonly in laboratories for disinfection of laboratory benches and as part of detergent germicides used for housekeeping purposes.

Low-level Disinfection

This procedure kills most vegetative bacteria except *M. tuberculosis*, some fungi, and inactivates some viruses. The EPA approves chemical germicides used in this procedure in the US as “hospital disinfectants” or “sanitizers.”

Decontamination in the Microbiology Laboratory

Decontamination in the microbiology laboratory must be carried out with great care. In this arena, decontamination may entail disinfection of work surfaces, decontamination of equipment so it is safe to handle, or may require sterilization. Regardless of the method, the purpose of decontamination is to protect the laboratory worker, the environment, and anyone who enters the laboratory or handles laboratory products away from the laboratory. Reduction of cross-contamination in the laboratory is an added benefit.

Decontamination and Cleaning

Decontamination renders an area, device, item, or material safe to handle (i.e., safe in the context of being reasonably free from a risk of disease transmission). The primary objective is to reduce the level of microbial contamination so that infection transmission is eliminated. The decontamination process may be ordinary soap and water cleaning of an instrument, device, or area. In laboratory settings, decontamination of items, spent laboratory materials, and regulated laboratory wastes is often accomplished by a sterilization procedure such as steam autoclaving, perhaps the most cost-effective way of decontaminating a device or an item.

The presence of any organic matter necessitates longer contact time with a decontamination method if the item or area is not pre-cleaned. For example, a steam cycle used to sterilize pre-cleaned items is 20 minutes at 121°C. When steam sterilization is used to decontaminate items that have a high bio-burden and there is no pre-cleaning (i.e., infectious waste) the cycle is longer. Decontamination in laboratory settings often requires longer exposure times because pathogenic microorganisms may be protected from contact with the decontaminating agents.

Table 1. Descending Order of Resistance to Germicidal Chemicals

Bacterial Spores *Bacillus subtilis, Clostridium sporogenes*
▼
Mycobacteria *Mycobacterium tuberculosis* var. *bovis*, Nontuberculous mycobacteria
▼
Nonlipid or Small Viruses Poliovirus, Coxsackievirus, Rhinovirus
▼
Fungi *Trichophyton* spp., *Cryptococcus* spp., *Candida* spp.
▼
Vegetative Bacteria *Pseudomonas aeruginosa, Staphylococcus aureus*, *Salmonella choleraesuis*, Enterococci
▼
Lipid or Medium-size Viruses Herpes simplex virus, CMV, Respiratory syncytial virus, HBV, HCV, HIV, Hantavirus, Ebola virus

Note: There are exceptions to this list. *Pseudomonas* spp are sensitive to high-level disinfectants, but if they grow in water and form biofilms on surfaces, the protected cells can approach the resistance of bacterial spores to the same disinfectant. The same is true for the resistance to glutaraldehyde by some nontuberculous mycobacteria, some fungal ascospores of *Microascus cinereus* and *Cheatomium globosum,* and the pink pigmented *Methylobacteria*. Prions are also resistant to most liquid chemical germicides and are discussed in the last part of this section.

Chemical germicides used for decontamination range in activity from high-level disinfectants (i.e., high concentrations of sodium hypochlorite [chlorine bleach]), which might be used to decontaminate spills of cultured or concentrated infectious agents in research or clinical laboratories, to low-level disinfectants or sanitizers for general housekeeping purposes or spot decontamination of environmental surfaces in healthcare settings. Resistance of selected organisms to decontamination is presented in descending order in Table 1. If dangerous and highly infectious agents are present in a laboratory, the methods for decontamination of spills, laboratory equipment, BSC, or infectious waste are very significant and may include prolonged autoclave cycles, incineration or gaseous treatment of surfaces.

Decontamination of Large Spaces

Space decontamination is a specialized activity and should be performed by specialists with proper training and protective equipment.[8] Decontamination requirements for BSL-3 and BSL-4 laboratory space have an impact on the design of these facilities. The interior surfaces of BSL-3 laboratories must be water resistant in order for them to be easily cleaned and decontaminated. Penetrations in these surfaces should be sealed or capable of being sealed for decontamination purposes. Thus, in the BSL-3 laboratory, surface decontamination, not fumigation, is the primary means of decontaminating space. Care should be taken that penetrations in the walls, floors and ceilings are kept to a minimum and are "sight sealed." Verification of the seals is usually not required for most BSL-3 laboratories. The BSL-4 laboratory design requires interior surfaces that are water resistant AND sealed to facilitate fumigation. These seals must be tested and verified to ensure containment in order to permit both liquid disinfection and fumigation. Periodic fumigation is required in the BSL-4 suit laboratory to allow routine maintenance and certification of equipment. Procedures for decontamination of large spaces such as incubators or rooms are varied and influenced significantly by the type of etiologic agent involved, the characteristics of the structure containing the space, and the materials present in the space. The primary methods for space decontamination follow.

Formaldehyde—Paraformaldehyde

Formaldehyde gas at a concentration of 0.3 grams/cubic foot for four hours is often used for space decontamination. Gaseous formaldehyde can be generated by heating flake paraformaldehyde (0.3 grams per cubic foot) in a frying pan, thereby converting it to formaldehyde gas. The humidity must be controlled and the system works optimally at 80% relative humidity. This method is effective in killing microorganisms but toxicity issues are present.[1,9] Additional information on environmental and safety issues related to paraformaldehyde is available from the EPA Web site: *www.epa.gov/pesticides*.

Hydrogen Peroxide Vapor

Hydrogen peroxide can be vaporized and used for the decontamination of glove boxes as well as small room areas. Vapor phase hydrogen peroxide has been shown to be an effective sporicide at concentrations ranging from 0.5 mg/L to <10 mg/L. The optimal concentration of this agent is about 2.4 mg/L with a contact time of at least one hour. This system can be used to decontaminate glove boxes, walk in incubators and small rooms. An advantage of this system is that the end products (i.e., water) are not toxic. Low relative humidity can be used.[10-14]

Chlorine Dioxide Gas

Chlorine dioxide gas sterilization can be used for decontamination of laboratory rooms, equipment, glove boxes, and incubators. The concentration of gas at the

site of decontamination should be approximately 10 mg/L with contact time of one to two hours.

Chlorine dioxide possesses the bactericidal, virucidal and sporicidal properties of chlorine, but unlike chlorine, does not lead to the formation of trihalomethanes or combine with ammonia to form chlorinated organic products (chloramines). The gas cannot be compressed and stored in high-pressure cylinders, but is generated upon demand using a column-based solid phase generation system. Gas is diluted to the use concentration, usually between 10 and 30 mg/L. Within reasonable limits, a chlorine dioxide gas generation system is unaffected by the size or location of the ultimate destination for the gas. Relative humidity does need to be controlled and high humidities are optimal. Although most often used in closed sterilizers, the destination enclosure for the chlorine dioxide gas does not, in fact, need to be such a chamber. Because chlorine dioxide gas exits the generator at a modest positive pressure and flow rate, the enclosure also need not be evacuated and could be a sterility-testing isolator, a glove box or sealed BSC, or even a small room that could be sealed to prevent gas egress.[15] Chlorine dioxide gas is rapidly broken down by light; care must be taken to eliminate light sources in spaces to be decontaminated.

Decontamination of Surfaces

Liquid chemical germicides formulated as disinfectants may be used for decontamination of large areas. The usual procedure is to flood the area with a disinfectant for periods up to several hours. This approach is messy and with some of the disinfectants used represents a toxic hazard to laboratory staff. For example, most of the "high-level" disinfectants on the United States market are formulated to use on instruments and medical devices and not on environmental surfaces. Intermediate and low-level disinfectants are formulated to use on fomites and environmental surfaces but lack the potency of a high-level disinfectant. For the most part intermediate and low level disinfectants can be safely used and, as with all disinfectants, the manufacturer's instructions should be closely followed.[7] Disinfectants that have been used for decontamination include sodium hypochlorite solutions at concentrations of 500 to 6000 parts per million (ppm), oxidative disinfectants such as hydrogen peroxide and peracetic acid, phenols, and iodophors.

Concentrations and exposure times vary depending on the formulation and the manufacturer's instructions for use.[6,16] See Table 2 for a list of chemical germicides and their activity levels. A spill control plan must be available in the laboratory. This plan should include the rationale for selection of the disinfecting agent, the approach to its application, contact time and other parameters. Agents requiring BSL-3 and BSL-4 containment pose a high risk to workers and possibly to the environment and should be managed by well-informed professional staff trained and equipped to work with concentrated material.

Table 2. Activity Levels of Selected Liquid Germicides[a]

Procedure / Product	Aqueous Concentration	Activity Level
Sterilization		
glutaraldehyde	variable	
hydrogen peroxide	6 – 30%	
formaldehyde	6 – 8%	
chlorine dioxide	variable	
peracetic acid		
Disinfection		
glutaraldehyde	variable	high to intermediate
ortho-phthalaldehyde	0.5%	high
hydrogen peroxide	3 – 6%	high to intermediate
formaldehyde [b]	1 – 8%	high to low
chlorine dioxide	variable	high
peracetic acid	variable	high
chlorine compounds [c]	500 to 5000 ml/L free/available chlorine	Intermediate
alcohols (ethyl, isopropyl) [d]	70%	Intermediate
phenolic compounds	0.5 to 3%	intermediate to low
iodophor compounds [e]	30 – 50 mg/L free iodine up to 10,000 mg/L available iodine 0.1 – 0.2%	intermediate to low
quaternary ammonium compounds		low

[a] This list of chemical germicides centers on generic formulations. A large number of commercial products based on these generic components can be considered for use. Users should ensure that commercial formulations are registered with EPA or by the FDA.

[b] Because of the ongoing controversy of the role of formaldehyde as a potential occupational carcinogen, the use of formaldehyde is limited to certain specific circumstances under carefully controlled conditions (e.g., for the disinfection of certain hemodialysis equipment). There are no FDA cleared liquid chemical sterilant/disinfectants that contain formaldehyde.

[c] Generic disinfectants containing chlorine are available in liquid or solid form (e.g., sodium or calcium hypochlorite). Although the indicated concentrations are rapid acting and broad-spectrum (tuberculocidal, bactericidal, fungicidal, and virucidal), no proprietary hypochlorite formulations are formally registered with EPA or cleared by FDA. Common household bleach is an excellent and inexpensive source of sodium hypochlorite. Concentrations between 500 and 1000 mg/L chlorine are appropriate for the vast majority of uses requiring an intermediate level of germicidal activity; higher concentrations are extremely corrosive as well as irritating to personnel, and their use should be limited to situations where there is an excessive amount of organic material or unusually high concentrations of microorganisms (e.g., spills of cultured material in the laboratory).

[d] The effectiveness of alcohols as intermediate level germicides is limited because they evaporate rapidly, resulting in short contact times, and also lack the ability to penetrate residual organic material. They are rapidly tuberculocidal, bactericidal and fungicidal, but may vary in spectrum of virucidal activity (see text). Items to be disinfected with alcohols should be carefully pre-cleaned then totally submerged for an appropriate exposure time (e.g., 10 minutes).

[e] Only those iodophors registered with EPA as hard-surface disinfectants should be used, closely following the manufacturer's instructions regarding proper dilution and product stability. Antiseptic iodophors are not suitable to disinfect devices, environmental surfaces, or medical instruments.

Special Infectious Agent Issues

Transmissible Spongiform Encephalopathy Agents (Prions)

The major exception to the rule in the previous discussion of microbial inactivation and decontamination is the causative agent of CJD or other prion agents responsible for transmissible spongiform encephalopathies of the central nervous system in humans or animals. Studies show that prions are resistant to conventional uses of heat and/or chemical germicides for the sterilization of instruments and devices. (See Section IX.)

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Appendix C—Transportation of Infectious Substances

An infectious substance is a material known to contain or reasonably expected to contain a pathogen. A pathogen is a microorganism (including bacteria, viruses, rickettsiae, parasites, fungi) or other agent, such as a proteinaceous infectious particle (prion), that can cause disease in humans or animals. Infectious substances may exist as purified and concentrated cultures, but may also be present in a variety of materials, such as body fluids or tissues. Transportation of infectious substances and materials that are known or suspected to contain them are regulated as hazardous materials by the United State Department of Transportation (DOT), foreign governments, and the International Civil Aviation Organization, and their transportation is subject to regulatory controls. For transport purposes, the term “infectious substance” is understood to include the term “etiologic agent.”

Transportation Regulations

International and domestic transport regulations for infectious substances are designed to prevent the release of these materials in transit to protect the public, workers, property, and the environment from the harmful effects that may occur from exposure to these materials. Protection is achieved through rigorous packaging requirements and hazard communication. Packages must be designed to withstand rough handling and other forces experienced in transportation, such as changes in air pressure and temperature, vibration, stacking, and moisture. Hazard communication includes shipping papers, labels, markings on the outside of packagings, and other information necessary to enable transport workers and emergency response personnel to correctly identify the material and respond efficiently in an emergency situation. In addition, shippers and carriers must be trained on these regulations so they can properly prepare shipments and recognize and respond to the risks posed by these materials.

Select agents include infectious substances that have been identified by the CDC and the USDA as having the potential to pose a severe threat to public health and safety. Persons who offer for transportation or transport select agents in commerce in the United States must develop and implement security plans for such transportation. A security plan must include an assessment of the possible transportation security risks for materials covered by the security plan and specific measures to reduce or eliminate the assessed risks. At a minimum, a security plan must include measures to address those risks associated with personnel security, en route security, and unauthorized access.

Regulations

Department of Transportation. 49 CFR Part 171-180, Hazardous Materials Regulations. Applies to the shipment of infectious substances in commercial transportation within the United States. Information on these regulations is available at: *http://www.phmsa.dot.gov/hazmat.*

United States Postal Service (USPS). 39 CFR Part 20, International Postal Service (International Mail Manual), and Part 111, General Information on Postal Service (Domestic Mail Manual). Regulations on transporting infectious substances through the USPS are codified in Section 601.10.17 of the Domestic Mail Manual and Section 135 of the International Mail Manual. A copy of the Domestic and International Mail Manuals may be obtained from the U.S. Government Printing Office by calling Monday through Friday, 7:30 a.m. – 9:00 p.m. EST: (202) 512-1800; toll free (866) 512-1800; or at the USPS Web site: *http://bookstore.gpo.gov/*.

Occupational Health and Safety Administration (OSHA). 29 CFR Part 1910.1030, Occupational Exposure to Bloodborne Pathogens. These regulations provide minimal packaging and labeling for blood and body fluids when transported within a laboratory or outside of it. Information may be obtained from your local OSHA office or at the OSHA Web site: *http://www.osha.gov*.

Technical Instructions for the Safe Transport of Dangerous Goods by Air (Technical Instructions). International Civil Aviation Organization (ICAO). Applies to the shipment of infectious substances by air and is recognized in the United States and by most countries worldwide. A copy of these regulations may be obtained from the ICAO Document Sales Unit at (514) 954-8022, fax: (514) 954-6769; e-mail: sales_unit@icao.int; or from the ICAO Web site: *http://www.icao.int*.

Dangerous Goods Regulations. International Air Transport Association (IATA). These regulations are issued by an airline association, are based on the ICAO Technical Instructions, and are followed by most airline carriers. A copy of these regulations is available at: *http://www.iata.org/index.htm* or *http://www.who.int/en/* ; or by contacting the IATA Customer Care office at: telephone: +1 (514) 390 6726; fax: +1 (514) 874 9659; for Canada and USA (800) 716-6326 (toll free); Europe, Africa and Middle East +41 (22) 770 2751; fax: +41 (22) 770 2674; TTY: YMQTPXB, or e-mail: *custserv@iata.org*.

Transfers

Regulations governing the transfer of biological agents are designed to ensure that possession of these agents is in the best interest of the public and the nation. These regulations require documentation of personnel, facilities, justification of need and pre-approval of the transfer by a federal authority. The following regulations apply to this category:

Importation of Etiologic Agents of Human Disease. 42 CFR Part 71 Foreign Quarantine. Part 71.54 Etiological Agents, Hosts and Vectors. This regulation requires an import permit from the CDC for importation of etiologic agents, hosts or vectors of human disease. The regulation, application form, and additional guidance is available at the CDC Web site: *http://www.cdc.gov/od/eaipp*.

Completed application forms may be submitted to the CDC Etiologic Agent Import Permit Program by fax: (404) 718-2093, or by mail:

Centers for Disease Control and Prevention
Etiologic Agent Import Permit Program
1600 Clifton Road, N.E., Mailstop A-46
Atlanta, GA 30333

Importation of select agents or toxins into the U.S. also requires the intended recipient to be registered with the Select Agent Program and submit an APHIS/CDC Form 2 to obtain approval to import the select agent or toxin prior to each importation event (see 42 CFR 73 and/or 9 CFR 121). More information regarding select agents and toxins is available at: *www.selectagents.gov*.

Importation of Etiologic Agents of Livestock, Poultry and Other Animal Diseases and Other Materials Derived from Livestock, Poultry or Other Animal. 9 CFR Parts 122. Organisms and Vectors. The USDA, APHIS, Veterinary Services (VS) requires that a permit be issued prior to the importation or domestic transfer (interstate movement) of etiologic disease agents of livestock, poultry, other animals. Information may be obtained at (301) 734-5960, or from the USDA Web site: *http://www.aphis.usda.gov/animal_health*. Completed permit applications may be submitted electronically at: *http://www.aphis.usda.gov/permits/learn_epermits.shtml*; or by fax to (301) 734-3652; or by mail to:

USDA APHIS VS
National Center for Import and Export
4700 River Road
Unit 2, Mailstop 22, Cubicle 1A07
Riverdale, MD 20737

Importation of select agents into the United States also requires the intended recipient to be registered with the Select Agent Program and submit an APHIS/CDC Form 2 to obtain approval to import the select agent or toxin prior to each importation event (see 42 CFR 73 and/or 9 CFR 121). More information regarding select agents and toxins is available at: *http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml*.

Importation of Plant Pests 7 CFR Part 330. Federal Plant Pest Regulations; General; Plant Pests; Soil; Stone and Quarry Products; Garbage. This regulation requires a permit for movement into or through the United States, or interstate any plant pest or a regulated product, article, or means of conveyance in accordance with this part. Information can be obtained by calling (877) 770-5990 or at the USDA Web site: *http://www.aphis.usda.gov/permits*.

Export of Etiologic Agents of Humans, Animals, Plants and Related Materials; Department of Commerce (DoC); 5 CFR Parts 730 to 799. This regulation requires that exporters of a wide variety of etiologic agents of human, plant and animal

diseases, including genetic material, and products which might be used for culture of large amounts of agents, will require an export license. Information may be obtained by calling the DoC Bureau of Export Administration at (202) 482-4811, or at the DoC Web site: *http://www.ntis.gov/products/export-regs.aspx*; or at *http://www.access.gpo.gov/bis/index.html*; and *http://www.bis.doc.gov*.

Transfer of CDC Select Agents and Toxins. 42 CFR Part 73 Possession, Use, and Transfer of Select Agents and Toxins. The CDC regulates the possession, use, and transfer of select agents and toxins that have the potential to pose a severe threat to public health and safety. The CDC Select Agent Program registers all laboratories and other entities in the United States that possess, use, or transfer a select agent or toxin. Entities transferring or receiving select agents and toxins must be registered with the Select Agent Program and submit an APHIS/CDC Form 2 (see 42 CFR 73 and/or 9 CFR 121) to obtain approval prior to transfer of a select agent or toxin. The regulations, Select Agent Program forms, and additional guidance is available at the CDC Web site: *www.selectagents.gov*.

Transfer of USDA Select Agents and Toxins. 9 CFR Part 121 Possession, Use, and Transfer of Select Agents and Toxins. The USDA, APHIS, VS regulates the possession, use, and transfer of select agents and toxins that have the potential to pose a severe threat to animal health or animal products. The VS Select Agent Program oversees these activities and registers all laboratories and other entities in the U.S. that possess, use, or transfer a VS select agent or toxin. Entities transferring or receiving select agents and toxins must be registered with either the CDC or APHIS Select Agent Program, and submit an APHIS/CDC Form 2 (see 42 CFR 73 and/or 9 CFR 121) to obtain approval prior to transfer of a select agent or toxin. The regulations, Select Agent Program forms, and additional guidance is available at the APHIS Web site: *http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml*.

Transfer of USDA Plant Pests

The movement of Plant Pests is regulated under two distinct and separate regulations: (1) 7 CFR Part 331. Agricultural Bioterrorism Protection Act of 2002; Possession, Use, and Transfer of Biological Agents and Toxins; and (2) 7 CFR Part 330 Federal Plant Pest Regulations; General; Plant Pests; Soil; Stone and Quarry Products; Garbage. The regulation found at 7 CFR Part 331 requires an approved Transfer Form (APHIS/CDC Form 2) prior to importation, interstate, or intrastate movement of a Select Agent Plant Pest. In addition, under 7 CFR Part 330, the movement of a Plant Pest also requires a permit for movement into or through the United States, or interstate any plant pest or a regulated product, article, or means of conveyance in accordance with this part. Information can be obtained by calling (301) 734-5960 or at the USDA Web site: *http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml*.

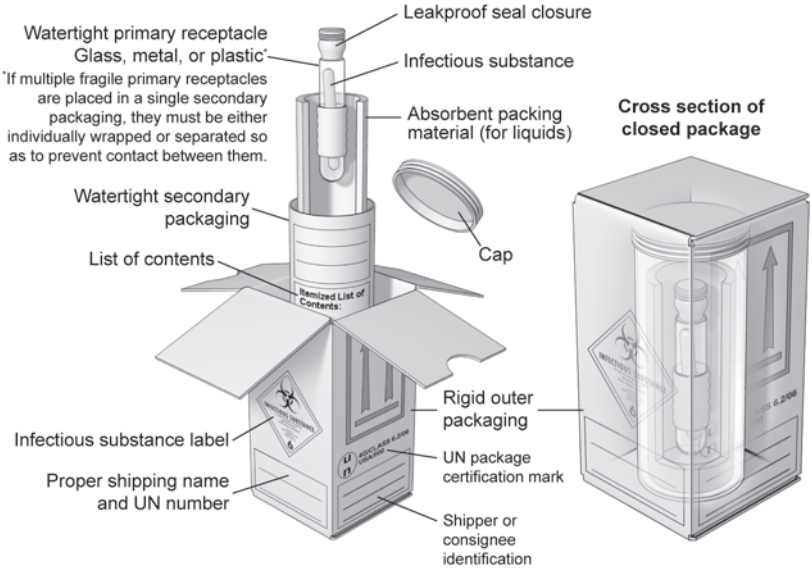
General DOT Packaging Requirements for Transport of Infectious Substances by Aircraft

The DOT packagings for transporting infectious substances by aircraft are required by domestic and international aircraft carriers, and are the basis for infectious substance packagings for motor vehicle, railcar, and vessel transport. The following is a summary of each packaging type and related transportation requirements.

Category A Infectious Substance (UN 2814 and UN 2900): Figure 1. A Category A material is an infectious substance that is transported in a form that is capable of causing permanent disability or life-threatening or fatal disease to otherwise healthy humans or animals when exposure to it occurs. An exposure occurs when an infectious substance is released outside of its protective packaging, resulting in physical contact with humans or animals. Category A infectious substances are assigned to identification number "UN 2814" for substances that cause disease in humans or in both humans and animals, or "UN 2900" for substances that cause disease in animals only.

Figure 1 shows an example of the UN standard triple packaging system for materials known or suspected of being a Category A infectious substance. The package consists of a watertight primary receptacle or receptacles; a watertight secondary packaging; for liquid materials, the secondary packaging must contain absorbent material in sufficient quantities to absorb the entire contents of all primary receptacles; and a rigid outer packaging of adequate strength for its capacity, mass, and intended use. Each surface of the external dimension of the packaging must be 100 mm (3.9 inches) or more. The completed package must pass specific performance tests, including a drop test and a water-spray test, and must be capable of withstanding, without leakage, an internal pressure producing a pressure differential of not less than 95 kPa (0.95 bar, 14 psi). The completed package must also be capable of withstanding, without leakage, temperatures in the range of -40ºC to +55ºC (-40ºF to 131ºF). The completed package must be marked "Infectious substances, affecting humans, UN 2814" or "Infectious substances, affecting animals, UN 2900" and labeled with a Division 6.2 (infectious substance) label. In addition, the package must be accompanied by appropriate shipping documentation, including a shipping paper and emergency response information.

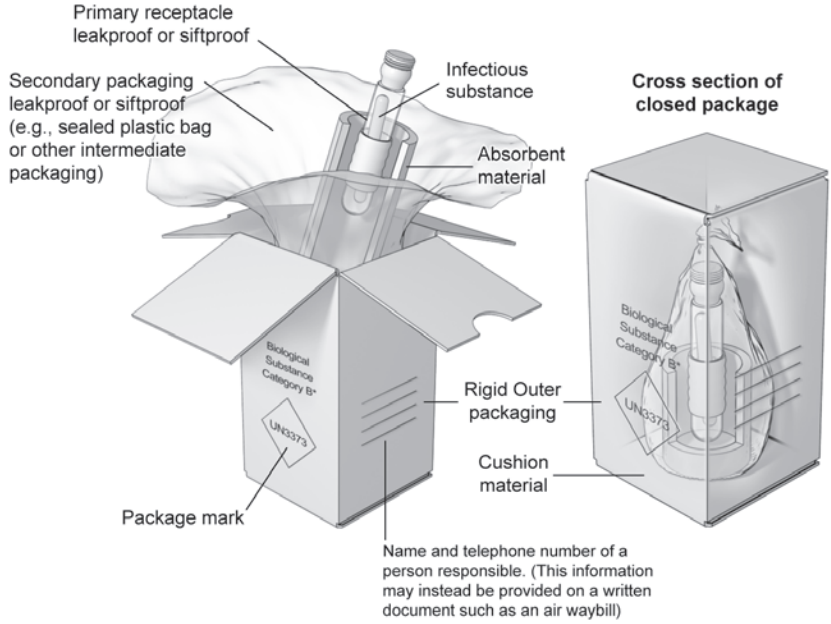
Figure 1. ***A Category A UN Standard Triple Packaging***



Biological specimen, Category B (UN 3373): Figure 2. (previously known as Clinical specimen and Diagnostic Specimen). A Category B infectious substance is one that does not meet the criteria for inclusion in Category A. A Category B infectious substance does not cause permanent disability or life-threatening or fatal disease to humans or animals when exposure to it occurs. The proper shipping name for a Category B infectious substance, "Biological specimen, Category B," is assigned to identification number "UN 3373." The proper shipping names "Diagnostic specimen" and "Clinical specimen" may no longer be used (as of January 1, 2007).

Figure 2 shows an example of the triple packaging system for materials known or suspected of containing a Category B infectious substance. A Category B infectious substance must be placed in a packaging consisting of a leak proof primary receptacle, leak proof secondary packaging, and rigid outer packaging. At least one surface of the outer packaging must have a minimum dimension of 100 mm by 100 mm (3.9 inches). The packaging must be of good quality and strong enough to withstand the shocks and loadings normally encountered during transportation. For liquid materials, the secondary packaging must contain absorbent material in sufficient quantities to absorb the entire contents of all primary receptacles. The primary or secondary packaging must be capable of withstanding, without leakage, an internal pressure producing a pressure differential of 95 kPa. The package must be constructed and closed to prevent any loss of contents that might be caused under normal transportation conditions by vibration or changes in temperature, humidity, or pressure. The completed package must be capable of passing a 1.2-meter (3.9 feet) drop test. The package must be marked with a diamond-shaped marking containing the identification number “UN 3373” and with the proper shipping name “Biological substance, Category B.” In addition, the name, address, and telephone number of a person knowledgeable about the material must be provided on a written document, such as an air waybill, or on the package itself.

Figure 2. ***A Category B Non-specification Triple Packaging***



Appendix D—Agriculture Pathogen Biosafety

The contents of this Appendix were provided by USDA. All questions regarding its contents should be forwarded to the USDA.

Contents

I. Introduction

Risk assessment and management guidelines for agriculture differ from human public health standards. Risk management for agriculture research is based on the potential economic impact of animal and plant morbidity, and mortality, and the trade implications of disease. Agricultural guidelines take this difference into account. Worker protection is important but great emphasis is placed on reducing the risk of agent escape into the environment. This Appendix describes the facility parameters and work practices of what has come to be known as BSL-3-Ag. BSL-3-Ag is unique to agriculture because of the necessity to protect the environment from an economic, high risk pathogen in a situation where studies are conducted employing large agricultural animals or other similar situations in which the ***facility barriers now serve as primary containment***. Also described are some of the enhancements beyond BSL-3 that may be required by USDA/APHIS when working in the laboratory or vivarium with veterinary agents of concern. This Appendix provides guidance and is not regulatory nor is it meant to describe policy. Conditions for approval to work with specific agricultural agents are provided at the time USDA/APHIS permits a location to work with an agent.

II. BSL-3-Ag for Work with Loose-housed Animals

In agriculture, special biocontainment features are required for certain types of research involving high consequence livestock pathogens in animal species or other research where the room provides the primary containment. To support such research, USDA has developed a special facility designed, constructed and operated at a unique animal containment level called BSL-3-Ag. Using the containment features of the standard ABSL-3 facility as a starting point, BSL-3-Ag facilities are specifically designed to protect the environment by including almost all of the features ordinarily used for BSL-4 facilities as enhancements. All BSL-3-Ag containment spaces must be designed, constructed and certified as primary containment barriers.

The BSL-3-Ag facility can be a separate building, but more often, it is an isolated zone within a facility operating at a lower biosafety level, usually at BSL-3. This isolated zone has strictly controlled access with special physical security measures and functions on the “box within a box” principle. All BSL-3-Ag facilities that cannot readily house animals in primary containment devices require the features for an ABSL-3 facility with the following enhancements typical of BSL-4 facilities:

1. Personnel change and shower rooms that provide for the separation of laboratory clothing from animal facility clothing and that control access to the containment spaces. The facility is arranged so that personnel ingress and egress are only through a series of rooms consisting of: a ventilated vestibule with compressible gaskets on the two doors, a “clean” change room outside containment, a shower room at the non-containment/containment boundary, and a “dirty” change room within containment. Complete animal facility clothing (including undergarments, pants and shirts or jump suits, and shoes and gloves) is typically provided in the “dirty” change room, and put on by personnel before entering the research areas. In some facilities, complete animal facility clothing and personal protective equipment are provided in the “clean” change room, where they can be stored and stowed for use without entry into containment. When leaving a BSL-3-Ag animal space that acts as the primary barrier and that contains large volumes of aerosols containing highly infectious agents (an animal room, necropsy room, carcass disposal area, contaminated corridor, etc.), personnel usually would be required to remove “dirty” lab clothing, take a shower, and put on “clean” lab clothing immediately after leaving this high risk animal space and before going to any other part of the BSL-Ag facility. When leaving the facility, these personnel would take another shower at the access control shower and put on their street clothing. Soiled clothing worn in a BSL-3-Ag space is autoclaved before being laundered. Personnel moving from one space within containment to another will follow the practices and procedures described in the biosafety manual specifically developed for the particular facility and adopted by the laboratory director.

2. Access doors are self closing and lockable. Emergency exit doors are provided, but are locked on the outside against unauthorized use. The architect or engineer shall consider the practicality of providing vestibules at emergency exits.

3. Supplies, materials and equipment enter the BSL-3-Ag space only through an airlock, fumigation chamber, an interlocked and double-door autoclave or shower.

4. Double-door autoclaves engineered with bioseals are provided to decontaminate laboratory waste passing out of the containment area. The double doors of the autoclaves must be interlocked so that the outer door can be opened only after the completion of the sterilizing cycle, and to prevent the simultaneous opening of both doors. All double door autoclaves are situated through an exterior wall of the containment area, with the autoclave unit forming an airtight seal with the barrier wall and the bulk of the autoclave situated outside the containment space so that autoclave maintenance can be performed conveniently. A gas sterilizer, a pass-through liquid dunk tank, or a cold gas decontamination chamber must be provided for the safe removal of materials and equipment that are steam or heat sensitive. Disposable materials must be decontaminated through autoclaving or other validated decontamination method followed by incineration.

5. Dedicated, single pass, directional, and pressure gradient ventilation systems must be used. All BSL-3-Ag facilities have independent air supply and exhaust systems that are operated to provide directional airflow and a negative air pressure within the containment space. The directional airflow within the containment spaces moves from areas of least hazard potential toward areas of greatest hazard potential. A visible means of displaying pressure differentials is provided. The pressure differential display/gauge can be seen inside and outside of the containment space, and an alarm sounds when the preset pressure differential is not maintained. The air supply and exhaust systems must be interlocked to prevent reversal of the directional airflow and positive pressurization of containment spaces in the event of an exhaust system failure.

6. Supply and exhaust air to and from the containment space is HEPA filtered. Exhaust air is discharged in such a manner that it cannot be drawn into outside air intake systems. The HEPA filters are outside of containment but are located as near as possible to the containment space to minimize the length of potentially contaminated air ducts. The HEPA filter housings are fabricated to permit scan testing of the filters in place after installation, and to permit filter decontamination before removal. Backup HEPA filter units are strongly recommended to allow filter changes without disrupting research. The most severe requirements for these modern, high level biocontainment facilities include HEPA filters arranged both in series and in parallel on the exhaust side, and parallel HEPA filters on the supply side of the HVAC systems serving “high risk” areas where large amounts of aerosols containing BSL-3-Ag agents could be expected (e.g., animal rooms, contaminated corridors, necropsy areas, carcass disposal facilities). For these high-risk areas, redundant supply and exhaust fans are recommended. The supply and exhaust air systems should be equipped with pre-filters (80-90% efficient) to prolong

the life of the HEPA filters. Air handling systems must provide 100% outside conditioned air to the containment spaces.

7. Liquid effluents from BSL-3-Ag areas must be collected and decontaminated in a central liquid waste sterilization system before disposal into the sanitary sewers. Typically, a heat decontamination system is utilized in these facilities and equipment must be provided to process, heat and hold the contaminated liquid effluents to temperatures, pressures and times sufficient to inactivate all biohazardous materials that reasonably can be expected to be studied at the facility in the future. The system may need to operate at a wide range of temperatures and holding times to process effluents economically and efficiently. Double containment piping systems with leak alarms and annular space decontaminating capability should be considered for these wastes. Effluents from laboratory sinks, cabinets, floors and autoclave chambers are sterilized by heat treatment. Under certain conditions, liquid wastes from shower rooms and toilets may be decontaminated by chemical treatment systems. Facilities must be constructed with appropriate basements or piping tunnels to allow for inspection of plumbing systems.

8. Each BSL-3-Ag containment space shall have its interior surfaces (walls, floors, and ceilings) and penetrations sealed to create a functional area capable of being certified as airtight. It is recommended that a pressure decay test be used (new construction only). Information on how to conduct a pressure decay test may be found within Appendix 9B of the ARS Facilities Design Manual (Policy and Procedure 242.1M-ARS; *http://www.afm.ars.usda.gov/*). This requirement includes all interior surfaces of all animal BSL-3-Ag spaces, not just the surfaces making up the external containment boundary. All walls are constructed slab to slab, and all penetrations, of whatever type, are sealed airtight to prevent escape of contained agents and to allow gaseous fumigation for biological decontamination. This requirement prevents cross contamination between individual BSL-3-Ag spaces and allows gaseous fumigation in one space without affecting other spaces. Exterior windows and vision panels, if required, are breakage-resistant and sealed. Greenhouses constructed to meet the BSL-3-Ag containment level will undergo the following tests, or the latest subsequent standards: (a) an air infiltration test conducted according to ASTM E 283-91; (b) a static pressure water resistance test conducted according to ASTM E 331-93; and (c) a dynamic pressure water resistance test conducted according to AAMA 501.1-94.

9. All ductwork serving BSL-3-Ag spaces shall be airtight (pressure tested-consult your facility engineer for testing and certification details).

10. The hinges and latch/knob areas of all passage doors shall be sealed to airtight requirements (pressure decay testing).

11. All airlock doors shall have air inflated or compressible gaskets. The compressed air lines to the air inflated gaskets shall be provided with HEPA filters and check valves.

12. Restraining devices shall be provided in large animal rooms.

13. Necropsy rooms shall be sized and equipped to accommodate large farm animals.

14. 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.

15. HEPA filters must be installed on all atmospheric vents serving plumbing traps, as near as possible to the point of use, or to the service cock, of central or local vacuum systems, and on the return lines of compressed air systems. All HEPA filters are installed to allow in-place decontamination and replacement. All traps are filled with liquid disinfectant.

16. If BSCs are installed, they should be located such that their operation is not adversely affected by air circulation and foot traffic. Class II BSCs use HEPA filters to treat their supply and exhaust air. Selection of the appropriate type of Class II BSCs will be dependent upon the proposed procedures and type of reagents utilized. BSC selection should be made with input from a knowledgeable safety professional well versed on the operational limitations of class II biohazard cabinetry. Supply air to a Class III cabinet is HEPA filtered, and the exhaust air must be double filtered (through a cabinet HEPA and then through a HEPA in a dedicated building exhaust system) before being discharged to the atmosphere.

III. BSL-3 and ABSL-3 Plus Potential Facility Enhancements for Agriculture Agent Permitting

The descriptions and requirements listed above for BSL-3-Ag studies are based on the use of high-risk organisms in animal systems or other types of agriculture research where the facility barriers, usually considered secondary barriers, now act as primary barriers. Certain agents that typically require a BSL-3-Ag facility for research that utilizes large agricultural animals may be studied in small animals in an enhanced BSL-3 laboratory or enhanced ABSL-3 when the research is done within primary containment devices. In these situations, the facility no longer serves as the primary barrier as with the large animal rooms. Therefore, when manipulating high consequence livestock pathogens in the laboratory or small animal facility, facility design and work procedures must meet the requirements of BSL-3 or ABSL-3 with additional enhancements unique to agriculture. Agriculture enhancements are agent, site and protocol dependent. The facility may have personnel

enter and exit through a clothing change and shower room, have a double-door autoclave and/or fumigation chamber, HEPA filter supply and exhaust air, and a validated or approved system in place to decontaminate research materials and waste. Surfaces must be smooth to support wipe-down decontamination and penetrations should be sealed and the room capable of sealing in case gaseous decontamination is required. Because all work with infectious material is conducted within primary containment, there is no requirement for pressure decay testing the room itself.

The need for any potential agriculture enhancements is dependant upon a risk assessment. Therefore, after an assessment and in consultation with USDA/ APHIS, the required agriculture enhancement(s) may include:

1. Personnel change and shower rooms that provide for the separation of street clothing from laboratory clothing and that control access to the containment spaces. The facility is arranged so that personnel ingress and egress are only through a series of rooms (usually one series for men and one for women) consisting of: a ventilated vestibule with a "clean" change room outside containment, a shower room at the non-containment/containment boundary, and a "dirty" change room within containment. Complete laboratory clothing (including undergarments, pants and shirts or jump suits, and shoes and gloves) is provided in the "dirty" change room, and put on by personnel before entering the research areas. In some facilities, complete laboratory clothing and personal protective equipment are provided in the "clean" change room, where they can be stored and stowed for use without entry into containment. When leaving a BSL-3 enhanced space, personnel usually would be required to remove their "dirty" laboratory clothing, take a shower, and put on "clean" laboratory clothing immediately after leaving the BSL-3 enhanced space and before going to any other part of the facility. Soiled clothing worn in a BSL-3 enhanced space should be autoclaved before being laundered outside of the containment space. Personnel moving from one space within containment to another will follow the practices and procedures described in the biosafety manual specifically developed for the particular facility and adopted by the laboratory director.

2. Access doors to these facilities are self closing and lockable. Emergency exit doors are provided but are locked on the outside against unauthorized use. The architect or engineer shall consider the practicality of providing vestibules at emergency exits.

3. Supplies, materials and equipment enter the BSL-3 enhanced space only through the double-door ventilated vestibule, fumigation chamber or an interlocked and double-door autoclave.

4. Double-door autoclaves engineered with bioseals are provided to decontaminate laboratory waste passing out of the containment area. The double doors of the autoclaves must be interlocked so that the outer door can be opened only after the completion of the sterilizing cycle, and to prevent the simultaneous opening of both doors. All double door autoclaves are situated through an exterior wall of the containment area, with the autoclave unit forming an airtight seal with the barrier wall and the bulk of the autoclave situated outside the containment space so that autoclave maintenance can be performed conveniently. A gas sterilizer, a pass-through liquid dunk tank, or a cold gas decontamination chamber must be provided for the safe removal of materials and equipment that are steam or heat sensitive. All other materials must be autoclaved or otherwise decontaminated by a method validated to inactivate the agent before being removed from the BSL-3 enhanced space. Wastes and other materials being removed from the BSL-3 enhanced space must be disposed of through incineration or other approved process.

5. Dedicated, single pass, directional, and pressure gradient ventilation systems must be used. All BSL-3 enhanced facilities have independent air supply and exhaust systems operated to provide directional airflow and a negative air pressure within the containment space. The directional airflow within the containment spaces moves from areas of least hazard potential toward areas of greatest hazard potential. A visible means of displaying pressure differentials is provided. The pressure differential display/gauge can be seen inside and outside of the containment space, and an alarm sounds when the preset pressure differential is not maintained. Supply and exhaust air to and from the containment space is HEPA filtered, with special electrical interlocks to prevent positive pressurization during electrical or mechanical breakdowns.

6. The exhaust air is discharged in such a manner that it cannot be drawn into outside air intake systems. HEPA filters located outside of the containment barrier are located as near as possible to the containment space to minimize the length of potentially contaminated air ducts. The HEPA filter housings are fabricated to permit scan testing of the filters in place after installation, and to permit filter decontamination before removal. Backup parallel HEPA filter units are strongly recommended to allow filter changes without disrupting research. Air handling systems must provide 100% outside conditioned air to the containment spaces.

7. Contaminated liquid wastes from BSL-3 enhanced areas must be collected and decontaminated by a method validated to inactivate the agent being used before disposal into the sanitary sewers. Treatment requirement will be determined by a site-specific, agent-specific risk assessment. Floor drains are discouraged in ABSL-3 and BSL-3

agriculture enhanced laboratories lacking a liquid waste central sterilization system. If floor drains are present, they should be capped and sealed. Facilities should be constructed with appropriate basements or piping tunnels to allow for inspection of plumbing systems, if a central liquid waste sterilization system is used.

8. Each BSL-3 enhanced containment space shall have its interior surfaces (walls, floors, and ceilings) and penetrations sealed to create a functional area capable of being decontaminated using a gaseous or vapor phase method. All walls are contiguous with the floor and ceiling, and all penetrations, of whatever type, are sealed. Construction materials should be appropriate for the intended end use. Exterior windows and vision panels, if required, are breakage-resistant and sealed.

9. All exhaust ductwork prior to the HEPA exhaust filter serving BSL-3 enhanced spaces shall be subjected to pressure decay testing before acceptance of the facility for use. Consult your facility engineer for testing and commissioning details.

IV. Pathogens of Veterinary Significance

Some pathogens of livestock, poultry and fish may require special laboratory design, operation, and containment features. This may be BSL-3, BSL-3 plus enhancements or BSL-4 and for animals ABSL-2, ABSL-3 or BSL-3-Ag. The importation, possession, or use of the following agents is prohibited or restricted by law or by USDA regulations or administrative policies.

This Appendix does not cover manipulation of diagnostic samples; however, if a foreign animal disease agent is suspected, samples should be immediately forwarded to a USDA diagnostic laboratory (The National Veterinary Services Laboratories, Ames, IA or the Foreign Animal Disease Diagnostic Laboratory, Plum Island, NY). A list of agents and their requirements follows.

African horse sickness virus [a, b]	Louping ill virus [a]
African swine fever virus [a, b, c]	Lumpy skin disease virus [a, b, c]
Akabane virus [b]	Malignant catarrhal fever virus (exotic strains or alcelaphine herpesvirus type 1) [b]
Avian influenza virus (highly pathogenic) [a, b, c]	Menangle virus [b]
Bacillus anthracis [a, b]	*Mycobacterium bovis*
Besnoitia besnoiti	*Mycoplasma agalactiae*
Bluetongue virus (exotic) [a, b]	*Mycoplasma mycoides subsp. mycoides* (small colony type) [a, b, c]

Borna disease virus	*Mycoplasma capricolum* [a, b, c]
Bovine infectious petechial fever agent	Nairobi sheep disease virus (Ganjam virus)
Bovine spongiform encephalopathy prion [b]	Newcastle disease virus (velogenic strains) [a, b, c]
Brucella abortus [a, b]	Nipah virus [a, b, d]
Brucella melitensis [a, b]	Peste des petits ruminants virus (plague of small ruminants) [a, b, c]
Brucella suis [a, b]	Rift Valley fever virus [a, b, c]
Burkholderia mallei/Pseudomonas mallei (Glanders) [a, b]	Rinderpest virus [a, b, c]
Burkholderia pseudomallei [a, b]	Sheep pox virus [a, b]
Camelpox virus [b]	Spring Viremia of Carp virus
Classical swine fever virus [a, b, c]	Swine vesicular disease virus [b]
Coccidioides immitis [b]	Teschen disease virus [a]
Cochliomyia hominivorax (Screwworm)	*Theileria annulata*
Coxiella burnetti (Q fever) [b]	*Theileria lawrencei*
Ephemeral fever virus	*Theileria bovis*
Ehrlichia (Cowdria) ruminantium (heartwater) [b]	*Theileria hirci*
Eastern equine encephalitis virus [a, b]	*Trypanosoma brucei*
Foot and mouth disease virus [a, b, c]	*Trypanosoma congolense*
Francisella tularensis [b]	*Trypanosoma equiperdum* (dourine)
Goat pox [a, b]	*Trypanosoma evansi*
Hemorrhagic disease of rabbits virus	*Trypanosoma vivax*
Hendra virus [a, b, d]	Venezuelan equine encephalomyelitis virus [a, b]
Histoplasma (Zymonema) farciminosum	Vesicular exanthema virus
Infectious salmon anemia virus	Vesicular stomatitis virus (exotic) [a, b]
Japanese encephalitis virus [a, b]	Wesselsbron disease virus

Notes:

[a] Export license required by Department of Commerce (See: *http://www.bis.doc.gov/index.htm*).

[b] Agents regulated as Select Agents under the Bioterrorism Act of 2002. Possession of these agents requires registration with either the CDC or APHIS and a permit issued for interstate movement or importation by APHIS-VS. Most require BSL-3/ABSL-3 or higher containment (enhancements as described in this Appendix or on a case-by-case basis as determined by APHIS-VS).

[c] Requires BSL-3-Ag containment for all work with the agent in loose-housed animals.

[d] Requires BSL-4 containment for all work with the agent.

A USDA/APHIS import or interstate movement permit is required to obtain any infectious agent of animals or plants that is regulated by USDA/APHIS. An import permit is also required to import any livestock or poultry product such as blood, serum, or other tissues.

V. Summaries of Selected Agriculture Agents

African Swine Fever Virus (ASFV)

ASF is a tick-borne and contagious, febrile, systemic viral disease of swine.[1,2,3] The ASF virus (ASFV) is a large (about 200 nm) lipoprotein-enveloped, icosahedral, double-stranded DNA virus in the family *Asfarviridae*, genus *Asfivirus*. This virus is quite stable and will survive over a wide range of pH. The virus will survive for 15 weeks in putrefied blood, three hours at 50ºC, 70 days in blood on wooden boards, 11 days in feces held at room temperature, 18 months in pig blood held at 4ºC, 150 days in boned meat held at 39ºF, and 140 days in salted dried hams. Initially, domestic and wild pigs (Africa: warthog, bush pig, and giant forest hog; Europe: feral pig) were thought to be the only hosts of ASFV. Subsequently, researchers showed that ASFV replicates in *Ornithodoros* ticks and that there is transstadial, transovarial, and sexual transmission. ASF in wild pigs in Africa is now believed to cycle between soft ticks living in warthog burrows and newborn warthogs. *Ornithodoros* ticks collected from Haiti, the Dominican Republic, and southern California have been shown to be capable vectors of ASFV, but in contrast to the African ticks, many of the ticks from California died after being infected with ASFV. Because ASFV-infected ticks can infect pigs, ASFV is the only DNA virus that can qualify as an arbovirus.

Even though the soft tick has been shown to be a vector (and in Africa probably the reservoir of ASFV), the primary method of spread from country to country has been through the feeding of uncooked garbage containing ASFV-infected pork scraps to pigs.

Aerosol transmission is not important in the spread of ASF. Because ASFV does not replicate in epithelial cells, the amount of virus shed by an ASF-infected pig is much less than the amount of virus shed by a hog-cholera-infected pig. The blood of a recently infected pig contains a very high ASFV titer.

Laboratory Safety and Containment Recommendations

Humans are not susceptible to ASFV infection. The greatest risk of working with the virus is the escape of the organism into a susceptible pig population, which would necessitate USDA emergency procedures to contain and eradicate the disease.

ASF is considered a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a

USDA-approved BSL-3-Ag facility for loosely housed animals. Special consideration should be given to infected vector control.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

African Horse Sickness Virus (AHSV)

AHSV is a member of genus *Orbivirus* in the family *Reoviridae*. Nine serotypes, numbers 1 – 9, are recognized. AHSV grows readily in embryonated chicken eggs, suckling mice, and a variety of standard cell cultures. AHSV infects and causes viremia in equids. Most horses die from the disease, about half of donkeys and most mules survive, but zebras show no disease. Viremias may last up to one month despite the rapid development of neutralizing antibodies. AHSV may cause disease in dogs, but these are not thought to be important in the natural history of the disease.[4,5]

AHSV has been recognized in central Africa and periodically spreads to naive populations in South and North Africa, the Iberian Peninsula, the Middle East, Pakistan, Afghanistan, and India. AHSV is vectored by *Culicoides* species and perhaps by mosquitoes, biting flies, and ticks limiting viral spread to climates and seasons favorable to the vectors. At least one North American *Culicoides* species transmits AHSV. AHSV may infect carnivores that consume infected animals but these are not thought to be relevant to natural transmission to equids.

Occupational Infections

Encephalitis and uveochorioretinitis were observed in four laboratory workers accidentally exposed to freeze-dried modified live vaccine preparations. Although AHSV could not be conclusively linked to disease, all four had neutralizing antibodies. Encephalitis was documented in experimentally infected monkeys.

Laboratory Safety and Containment Recommendations

Virus may be present in virtually any sample taken from an infected animal, but the highest concentrations are found in spleen, lung, and lymph nodes. The only documented risk to laboratory workers involves aerosol exposure to large amounts of vaccine virus. AHSV is unusually stable in blood or serum stored at 4°C.

AHS is considered a foreign animal disease in the United States. Due to the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with

enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements. Blood, serum, or tissues taken from equids in areas where AHSV exists are potential means of transmitting the agent long distances. Special consideration should be given to infected vector containment.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Akabane Virus (AKAV)

AKAV is a member of the genus *Orthobunyavirus* in the Simbu serogroup of the family *Bunyaviridae*. The Simbu serogroup also includes Aino, Peaton, and Tinaroo viruses that can cause similar disease. Experimental infection of pregnant hamsters leads to death of the fetus. This virus grows and causes disease in chick embryos. Isolated in suckling mice and hamster lung cell cultures, AKAV is an important cause of disease in ruminants. The virus does not cause overt disease in adults but infects the placenta and fetal tissues in cattle, sheep, and goats to cause abortions, stillbirths, and congenital malformations. The broad range of clinical signs in the fetus is related primarily to central nervous system damage that occurs during the first trimester of pregnancy.[6,7]

AKAV is not known to infect or cause disease in humans; concern focuses only on effects to agriculture and wildlife. Common names of disease include congenital arthrogryposis-hydranencephaly syndrome, Akabane disease, acorn calves, silly calves, curly lamb disease, curly calf disease, and dummy calf disease. The host range of naturally occurring Akabane disease appears limited to cattle, sheep, swine, and goats but other animals including swine and numerous wildlife species become infected. AKAV is an Old World virus, being found in Africa, Asia, and Australia. Disease is unusual in areas where the virus is common because animals generally become immune before pregnancy. AKAV spreads naturally only in gnat and mosquito insect vectors that become infected after feeding on viremic animals.

Laboratory Safety and Containment Recommendations

AKAV may be present in blood, sera, and tissues from infected animals, as well as vectors from endemic regions. Parenteral injection of these materials into naive animals and vector-borne spread to other animals represents a significant risk to agricultural interests.

Akabane disease is considered a foreign animal disease in the United States. Due to the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements. Special consideration should be given to infected vector containment.

Specials Issues

Although it is virtually certain AKAV will grow and cause disease in New World livestock, it is not known if it will cause viremias in New World wildlife high enough to infect vectors, if it can be vectored by New World insects, or if it will cause disease in New World wildlife. Because fetal disease may not become evident until months after virus transmission, an introduction into a new ecosystem may not be recognized before the virus has become firmly entrenched.

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Bluetongue Virus (BTV)

BTV is a member of the family *Reoviridae*, genus *Orbivirus*. There are 24 recognized serotypes numbered 1 through 24. BTV is notable for causing disease in sheep and cattle and is very similar to other orbiviruses that cause disease in deer (epizootic hemorrhagic disease of deer virus) and horses (AHSV), and a few that cause disease in man (Colorado tick fever virus and others). These viruses have dsRNA genomes distributed amongst 10 segments, enabling efficient reassortment. Growth on a wide variety of cultured cells is usually cytocidal. Growth in animals results in viremia within three to four days that endures as long as 50 days despite the presence of high levels of neutralizing antibodies.[8,9]

BTV infects all ruminants, but bluetongue disease is unusual except in sheep and is unpredictable even in sheep. Disease is evidenced by fever, hyperemia, swelling, and rarely erosions and ulceration of the buccal and nasal mucosa. Hyperemia of the coronary bands of the hooves may cause lameness. In the worst cases, the disease progresses through weakness, depression, rapid weight loss, prostration, and death. Maternal transmission to the fetus may cause abortion or fetal abnormalities in the first trimester. Bluetongue disease also occurs in cattle but is rarely diagnosed. BTV may infect fetal calves and result in abortion or fetal brain damage. The full host range of BTV is still unknown but includes wild ruminants, neonatal mice, dogs, and chicken embryos.

BTV infection occurs in tropical, subtropical, and temperate climates where the *Culicoides* vectors exist. Global warming may be expanding the geographic range of *Culicoides*, and therefore BTV, into higher latitudes. Most countries have a unique assortment of the 24 serotypes. For example, BTV serotypes 2, 10, 11, 13, and 17 are currently active in the United States, but serotypes 1, 3, 4, 6, 8, 12, and 17 were present in the Caribbean basin when last surveyed. Concern over the spread of individual serotypes by trade in animals and animal products has engendered costly worldwide trade barriers.

The primary natural mode of transmission is by *Culicoides* midges. Only a few of more than 1,000 species of *Culicoides* transmit BTV. A strong correlation between the vector species and the associated BTV suggests these viruses may have adapted to their local vector. Thus, BTV does not exist in areas such as the Northeast United States where the local *Culicoides* fails to transmit BTV. Virus is present in semen at peak of viremia, but this is not considered a major route of transmission. Because of the prolonged viremia, iatrogenic transmission is possible. Only modified-live (attenuated) virus vaccines are available and a vaccine for only one serotype is currently available in the United States.

Laboratory Safety and Containment Recommendations

BTV is not known to cause disease in humans under any conditions. BTV commonly enters the laboratory in blood samples. The virus is stable at -70°C and in blood or washed blood cells held at 4°C. Sera prepared from viremic animals may represent some risk if introduced parenterally into naive animals. Blood, sera, and bovine semen can carry BTV across disease control boundaries.

The most significant threat from BTV occurs when virus is inoculated parenterally into naive animals. If appropriate *Culicoides* are present, virus can be transmitted to other hosts. Therefore, BTV-infected animals must be controlled for the two-month period of viremia and protected against *Culicoides* by physical means and/ or performing experiments at least two months before local *Culicoides* emerge. Thus, BTV exotic to the United States should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 with enhancements. Special consideration should be given to infected vector containment. Special containment is only needed when working with serotypes of BTV that are exotic to the country or locality. BTV on laboratory surfaces is susceptible to 95% ethanol and 0.5% sodium hypochlorite solution.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Classical Swine Fever Virus (Hog Cholera)

Classical swine fever is a highly contagious viral disease of swine that occurs worldwide in an acute, a subacute, a chronic, or a persistent form.[10-12] In the acute form, the disease is characterized by high fever, severe depression, multiple superficial and internal hemorrhages, and high morbidity and mortality. In the chronic form, the signs of depression, anorexia, and fever are less severe than in the acute form, and recovery is occasionally seen in mature animals. Transplacental infection with viral strains of low virulence often results in persistently infected piglets, which constitute a major cause of virus dissemination to noninfected farms. Although minor antigenic variants of classical swine fever virus (CSFV) have been reported, there is only one serotype. Hog cholera virus is a lipid-enveloped pathogen belonging to the family *Flaviviridae*, genus *Pestivirus*. The organism has a close antigenic relationship with the bovine viral diarrhea virus (BVDV) and the border disease virus (BDV). In a protein-rich environment, hog cholera virus is very stable and can survive for months in refrigerated meat and for years in frozen meat. The virus is sensitive to drying (desiccation) and is rapidly inactivated by a pH of less than 3 and greater than 11.

The pig is the only natural reservoir of CSFV. Blood, tissues, secretions and excretions from an infected animal contain virus. Transmission occurs mostly by the oral route, though infection can occur through the conjunctiva, mucous membrane, skin abrasion, insemination, and percutaneous blood transfer (e.g., common needle, contaminated instruments). Airborne transmission is not thought to be important in the epizootiology of classical swine fever. Introduction of infected pigs is the principal source of infection in classical swine fever-free herds. Farming activities such as auction sales, livestock shows, visits by feed dealers, and rendering trucks also are potential sources of contagion. Feeding of raw or insufficiently cooked garbage is a potent source of hog cholera virus. During the warm season, insect vectors common to the farm environment may spread hog cholera virus mechanically. There is no evidence, however, that hog cholera virus replicates in invertebrate vectors.

Laboratory Safety and Containment Recommendations

Humans being are not susceptible to infection by CSFV. The greatest risk of working with these viruses is the escape of the organism into susceptible domestic or feral pig populations, which would necessitate USDA emergency procedures to contain and eradicate the diseases.

The virus is considered cause of a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed

animals. Laboratory workers should have no contact with susceptible hosts for five days after working with the agent.

Specials Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Contagious Bovine Pleuropneumonia Agent (CBPP)

CBPP is a highly infectious acute, subacute, or chronic disease, primarily of cattle, affecting the lungs and occasionally the joints, caused by *Mycoplasma mycoides mycoides*.[13-15] Contagious bovine pleuropneumonia is caused by *M. mycoides mycoides* small-colony type (SC type). *M. mycoides mycoides* large-colony type is pathogenic for sheep and goats but not for cattle. *M. mycoides mycoides* (SC type) survives well only *in vivo* and is quickly inactivated when exposed to normal external environmental conditions. The pathogen does not survive in meat or meat products and does not survive outside the animal in nature for more than a few days. Many of the routinely used disinfectants will effectively inactivate the organism.

CBPP is predominantly a disease of the genus *Bos*; both bovine and zebu cattle are naturally infected. There are many reported breed differences with respect to susceptibility. In general, European breeds tend to be more susceptible than indigenous African breeds. In zoos, the infection has been recorded in bison and yak. Although it has been reported that the domestic buffalo (*Bubalus bubalis*) is susceptible, the disease is difficult to produce experimentally in this species.

CBPP is endemic in most of Africa. It is a problem in parts of Asia, especially India and China. Periodically, CBPP occurs in Europe, and outbreaks within the last decade have occurred in Spain, Portugal, and Italy. The disease was eradicated from the United States in the nineteenth century, and it is not present currently in the Western hemisphere.

CBPP is spread by inhalation of droplets from an infected, coughing animal. Consequently, relatively close contact is required for transmission to occur. Outbreaks usually begin as the result of movement of an infected animal into a naive herd. There are limited anecdotal reports of fomite transmission, but fomites are not generally thought to be a problem.

Laboratory Safety and Containment Recommendations

Humans are not susceptible to infection by CBPP. The greatest risk of working with these mycoplasma is the escape of the organism into susceptible domestic bovine populations, which would necessitate USDA emergency procedures to contain and eradicate the diseases.

CBPP is considered a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Contagious Caprine Pleuropneumonia Agent (CCPP)

CCPP is an acute highly contagious disease of goats caused by a mycoplasma and characterized by fever, coughing, severe respiratory distress, and high mortality.[16-18] The principal lesion at necropsy is fibrinous pleuropneumonia. The causative agent of CCPP is considered to be *M. mycoides capri* (type strain PG-3) or a new mycoplasma *M. capricolum* subsp. *capripneumoniae* (designated F-38).[19-21] Neither of these agents occurs in North America.

M. mycoides mycoides has also been isolated from goats with pneumonia. This agent (the so-called large colony or LC variant of *M. mycoides mycoides*) usually produces septicemia, polyarthritis, mastitis, encephalitis, conjunctivitis, hepatitis, or pneumonia in goats. Some strains of this agent (LC variant) will cause pneumonia closely resembling CCPP, but the agent is not highly contagious and is not considered to cause CCPP. It does occur in North America. *M. capricolum capricolum*, a goat pathogen commonly associated with mastitis and polyarthritis in goats, can also produce pneumonia resembling CCPP, but it usually causes severe septicemia and polyarthritis. This agent (which does occur in the United States) is closely related to mycoplasma F-38 but can be differentiated from it using monoclonal antibodies.

CCPP is a disease of goats, and where the classical disease has been described, only goats were involved in spite of the presence of sheep and cattle. Mycoplasma F-38, the probable cause of the classic disease, does not cause disease in sheep or cattle. *M. mycoides capri*, the other agent considered a

cause of CCPP, will result in a fatal disease in experimentally inoculated sheep and can spread from goats to sheep. It is however, not recognized as a cause of natural disease in sheep.

CCPP has been described in many countries of Africa, the Middle East, Eastern Europe, the former Soviet Union, and the Far East. It is a major scourge in many of the most important goat-producing countries in the world and is considered by many to be the world's most devastating goat disease.

CCPP is transmitted by direct contact through inhalation of infective aerosols. Of the two known causative agents, F-38 is far more contagious. Outbreaks of the disease often occur after heavy rains (e.g., after the monsoons in India) and after cold spells. This is probably because recovered carrier animals start shedding the mycoplasmas after the stress of sudden climatic change. It is believed that a long-term carrier state may exist.

Laboratory Safety and Containment Recommendations

Humans are not susceptible to infection by the agent that causes CCPP. The greatest risk of working with this mycoplasma is the escape of the organism into susceptible domestic caprine populations, which would necessitate USDA emergency procedures to contain and eradicate the diseases.

CCPP is considered a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Foot and Mouth Disease Virus (FMD)

FMD is a severe, highly communicable viral disease of cloven-hoofed animals (cattle, swine, sheep, and goats), causing fever, malaise, vesicular lesions in affected livestock and in some cases death in young animals due to myocardial lesions.[22] It can also affect a variety of wild ruminants (e.g., deer, bison). FMD is one of the most devastating diseases of livestock, causing large economic losses when introduced to FMD-free countries. The etiologic agent, FMD virus (FMDV), is a member of the *aphtovirus* genus, family *picornaviridae* with seven serotypes

(A, O, C, Asia1, SAT1, SAT2 and SAT3).[23] Humans are considered accidental hosts for FMDV and rarely become infected or develop clinical disease. Historically, humans have been exposed to large quantities of FMDV both during natural outbreaks among large herds of animals and in laboratory settings. Despite this, there has been an extremely low incidence of human infections reported and many have been anecdotal. Reports of fever, headaches and vesicles in the skin (especially at an accidental inoculation site) and oral mucosa have been associated with documented FMDV infections. The symptoms can be easily mistaken with those of Hand, Foot and Mouth Disease caused by coxsackie A viruses. On the other hand, humans have been shown to carry virus in their throats for up to three days after exposure to aerosols from infected animals, potentially making them carriers of FMDV. Humans and their clothing and footwear have been implicated as fomites for transmission of FMDV during outbreaks. Therefore, most FMDV laboratories impose a five day period of contact avoidance with susceptible species for personnel working with the viruses.

Laboratory Safety and Containment Recommendations

Laboratory practices for FMDV are principally designed to prevent transmission to susceptible livestock, but also to protect workers. The greatest risk of working with FMD is the escape of the organism into susceptible animal populations, which would necessitate USDA emergency procedures to contain and eradicate the disease.

The virus is considered a cause of a foreign animal disease in the United States. Due to the highly contagious nature and the severe economic consequences of disease presence in the United States, this virus should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA (see Section IV of this Appendix) and *in vivo* in USDA-approved BSL-3-Ag animal facilities. Infected animals are handled with standard protection (gloves, protective clothing). Change of clothing, personal showers and clearing of the throat and nose are required upon exiting contaminated areas in order to minimize virus transmission to susceptible species. Laboratory workers should have no contact with susceptible hosts for five days after working with the agent. In the United States, the Plum Island Animal Disease Center in New York is the only laboratory authorized to possess and work with this agent.

Special Issues

FMDV is a select agent. Possession, transfer and use of this agent requires application of procedures as detailed in the Agricultural Bioterrorism Protection Act of 2002 and codified in 9 CFR Part 121. All rules concerning the possession, storage, use, and transfer of select agents apply. Please review Appendix F of this document for further instructions regarding select agents. Law prohibits research with FMD on the United States mainland.

Heartwater Disease Agent (HD)

HD is a non-contagious disease of domestic and wild ruminants caused by *Ehrlichia ruminantium*.[24] *E. ruminantium* (formerly *Cowdria ruminantium*) is a member of the family *Rickettsiaceae* characterized by organisms that are obligate intracellular parasites. These organisms often persist in the face of an immune response due to their protected intracellular status. Rickettsias in natural conditions are found in mammals and blood-sucking arthropods. Ticks of the genus *Amblyomma* transmit *E. ruminantium*. HD occurs primarily in Africa, but has been recognized in the West Indies since the 1980's. The pathogen is transmitted by ticks of the genus *Amblyomma*, most importantly *A. variegatum* (tropical bont tick). This tick has wide distribution in Africa and is present on several Caribbean islands. Three North American tick species, *A. maculatum* (Gulf Coast tick), *A. cajennese*, and *A. dissimile*, can transmit the organism, causing concern that competent vectors could transmit *E. ruminantium* in the United States.

Severe HD comprises fever, depression, rapid breathing, and convulsions in cattle, sheep, goats and water buffalo. Whitetail deer also are susceptible to *E. ruminantium* infection and develop severe clinical disease. HD has not been diagnosed in the United States but occurs in numerous Caribbean islands, as well as in most countries of Africa, south of the Sahara Desert.

Laboratory Safety and Containment Recommendations

E. ruminantium can be found in whole blood, brain and experimentally in liver and kidney. It is not a human pathogen. Humans are not susceptible to infection with the agent that causes HD. The greatest risk of working with this agent is the escape of this organism (or infected ticks) into a susceptible domestic bovine population, which would necessitate USDA emergency procedures to contain and eradicate the disease.

HD is considered a foreign animal disease in the United States. *E. ruminantium* should be handled *in vitro* in BSL-3 laboratory facilities. Animal work should be conducted in ABSL-3 animal facilities or in ABSL-2 animal facilities with special modifications such as tick dams (where applicable).

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Infectious Salmon Anemia (ISA) Virus

ISA is a disease of Atlantic salmon (*Salmo salar*) caused by an orthomyxovirus in the family *Orthomyxoviridae*, genus *Isavirus*. Both wild and cultured Atlantic salmon are susceptible to infection, as are brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) and herring. The first clinical cases of ISA in Atlantic salmon were reported from Norway in 1984. Since then, ISA has been observed in Canada (1996), Scotland (1998), Chile (1999), Faroe Islands (2000) and the U.S. (2001).[25,26] There is significant molecular difference between virus isolates (i.e., "Norwegian", "Scottish" and "North American").[27] Clinical signs of ISA include severe anemia, swelling and hemorrhaging in the kidney and other organs, pale gills, protruding eyes, darkening of the posterior gut, fluid in the body cavity and lethargy. The infection is systemic and most noted in blood and mucus, muscle, internal organs and feces. The principal target organ for ISA virus (ISAV) is the liver. Signs usually appear two to four weeks after the initial infection.

Reservoirs of ISAV infection are unknown, but the spread of infection may occur due to the purchase of subclinically infected smolts, from farm to farm, and from fish slaughterhouses or industries where organic material (especially blood and processing water) from ISAV-infected fish is discharged without necessary treatment.[28]

Laboratory Safety and Containment Recommendations

Humans are not susceptible to ISAV infection. The greatest risk of working with this virus is the escape of the organism into a susceptible fish population, which would necessitate USDA emergency procedures to contain and eradicate the disease.

ISA is considered a reportable disease in the United States. ISAV should be handled *in vitro* in BSL-2 laboratory facilities with enhancements as required by USDA. Animal inoculations should be handled in ABSL-3 animal facilities with special modifications as required. Recommended precautions include incineration of fish, tissues, blood and materials (gloves, laboratory coats, etc.) used in the collection and processing of fish samples. All surfaces exposed to potentially infected fish should be disinfected with 0.04 to 0.13% acetic acid, chlorine dioxide at 100 parts/million for five minutes or sodium hypochlorite 30 mg available chlorine/liter for two days or neutralized with sodium thiosulfate after three hours. General principles of laboratory safety should be practiced in handling and processing fish samples for diagnostic or investigative studies. Laboratory managers should evaluate the need to work with ISAV and the containment capability of the facility before undertaking work with the virus or suspected ISAV-infected fish.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or

interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Lumpy Skin Disease (LSD)Virus

LSD is an acute to chronic viral disease of cattle characterized by skin nodules that may have inverted conical necrosis (sit fast) with lymphadenitis accompanied by a persistent fever.[29-31] The causative agent of LSD is a capripoxvirus in the family *Poxviridae*, genus *Capripoxvirus*. The prototype strain of LSD virus (LSDV) is the Neethling virus. LSDV is one of the largest viruses (170-260 nm by 300-450 nm) and there is only one serotype. The LSDV is very closely related serologically to the virus of sheep and goat pox (SGP) from which it cannot be distinguished by routine virus neutralization or other serological tests. The virus is very resistant to physical and chemical agents, persists in necrotic skin for at least 33 days and remains viable in lesions in air-dried hides for at least 18 days at ambient temperature.

LSD is a disorder of cattle. Other wild ungulates have not been infected during epizootics in Africa. Lumpy skin disease was first described in Northern Rhodesia in 1929. Since then, the disease has spread over most of Africa in a series of epizootics and most recently into the Middle East. Biting insects play the major (mechanical) role in the transmission of LSDV. Direct contact seems to play a minor role in the spread of LSD.

Laboratory Safety and Containment Recommendations

Human beings are not susceptible to infection by LSDV. The greatest risk of working with this virus is the escape of the organism into susceptible domestic animal populations, which would necessitate USDA emergency procedures to contain and eradicate the diseases.

Lumpy skin disease is considered a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Malignant Catarrhal Fever Virus (MCFV) (Exotic Strains)

Alcelaphine herpesvirus 1 (AlHV-1*)* is a herpesvirus of the *Rhadinovirus* genus in the *Gammaherpesvirinae* subfamily.[32] Common names for AlHV-1 include wildebeest-associated malignant catarrhal fever virus (MCFV), African form MCFV, and exotic MCFV. It also was previously called bovine herpesvirus[3]. As a typical herpesvirus, AlHV-1 is a linear double-stranded DNA, enveloped virus. The virus can be propagated in certain primary or secondary cell cultures such as bovine thyroid and testis cells. The isolation of AlHV-1 requires the use of viable lymphoid cells from the diseased animal or cell-free virus in ocular/nasal secretions from wildebeest calves during a viral shedding period. Like other herpesviruses, AlHV-1 is fragile and quickly inactivated in harsh environments (for example, desiccation, high temperatures, and UV/sunlight), and by common disinfectants.

Wildebeest-associated MCF caused by AlHV-1 is also known as the African form of MCF, malignant catarrh, or snotsiekte (snotting sickness). The disease primarily affects many poorly adapted species of *Artiodactyla* that suffer very high case mortality (>95%) but low case morbidity (<7%). Wildebeest are the reservoir for AlHV-1 and the virus does not cause any significant disease in its natural host. Wildebeest-associated MCF primarily occurs in domestic cattle in Africa and in a variety of clinically susceptible ruminant species in zoological collections where wildebeest are present. Virtually all free-living wildebeest are infected with the virus and calves less than four months of age serve as the source of virus for transmission. The disease can be experimentally transmitted between cattle only by injection with infected viable cells from lymphoid tissues of affected animals. The disease cannot be transmitted by natural means from one clinically susceptible host to another, because there is essentially no cell-free virus in tissues or secretions of diseased animals. MCF is not a contagious disease.

Laboratory Safety and Containment Recommendations

There is no evidence that AlHV-1 can infect humans. Virus can be grown in several bovine cell lines at relatively low titers (ranging from 103 to 105 $TCID_{50}$). Infectivity in blood and tissues of affected animals is generally associated with viable lymphoid cells. The virus can be easily inactivated by wiping down surfaces with common disinfectants (such as bleach and sodium hypochlorite) and by autoclaving virus-contaminated materials.

This organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or

interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Menangle Virus (MenV)

MenV caused a single outbreak of reproductive disease in an Australian swine operation. Clinical signs included stillborn, deformed, mummified piglets and a drop in the farrowing rate. Transmission between pigs has been postulated to be of a fecal-oral nature. A serological survey of fruit bats living near the swine operation revealed the presence of antibodies to MenV. Fruit bats are considered to be the natural host of the virus and their proximity to the affected premises led to an incidental infection in the pig population.[33,34]

MenV is a member of the family *Paramyxoviridae*, subfamily *Paramyxovirinae*. Other members of this family include Hendra virus, Nipah virus and Tioman virus of which Hendra and Nipah have been found to be fruit bat-associated. This virus was isolated from stillborn piglets from a single outbreak of reproductive disease in a commercial swine operation in New South Wales, Australia in 1997.

Occupational Infections

There was serological evidence of MenV infection in two people that had close contact with infected pigs on the affected premises. They demonstrated clinical signs similar to those seen with influenza such as chills, fever, drenching sweats, headache and rash. Both workers recovered fully from their illness.

Laboratory Safety and Containment Recommendations

Laboratory practices for MenV are principally designed to prevent transmission to susceptible livestock, but also to protect workers. The greatest risk of working with MenV is the escape of the organism into susceptible animal populations, which would necessitate USDA emergency procedures to contain and eradicate the disease.

MenV is considered cause of a foreign animal disease in the United States and is a human pathogen. Due to the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Newcastle Disease (ND) Virus

ND is one of the most serious infectious diseases of poultry worldwide. It is primarily a respiratory disease, but nervous and enteric forms occur. All bird species are probably susceptible to infection with ND virus (NDV). The severity of the disease caused by any given NDV strain can vary from an unapparent infection to 100% mortality. The chicken is the most susceptible species. The biocontainment requirements for working with a particular strain are based on the virulence of the virus as determined by chicken inoculation and more recently by determination of amino acid sequence of the fusion protein cleavage site (as defined by the World Organization for Animal Health).[35] The virus is shed in respiratory secretions and in feces. Natural transmission among birds occurs by aerosol inhalation or by consumption of contaminated feed or water.[36,37]

NDV is classified in the *Avulavirus* genus within the family *Paramyxoviridae,* subfamily *Paramyxovirinae,* in the order *Mononegavirales*. All NDV isolates are of a single serotype avian paramyxovirus type 1 (APMV-1) that includes the antigenic variants isolated from pigeons called pigeon paramyxovirus[1]. All strains are readily propagated in embryonated chicken eggs and a variety of avian and mammalian cell cultures although special additives may be required to propagate the low virulence (lentogenic) viruses in some cell types.[35-37]

Occupational Infections

The most common infection is a self-limiting conjunctivitis with tearing and pain that develops within 24 hours of an eye exposure by aerosol, splash of infective fluids, or eye contact with contaminated hands. The occurrence of upper respiratory or generalized symptoms is rare.[38]

Laboratory Safety and Containment Recommendations

NDV isolates may be recovered from any infected bird, but on occasion may be recovered from humans infected by contact with infected poultry. Humans treated with live NDV in experimental cancer therapies, or those who are exposed by laboratory contamination also are sources of the virus.[38] The greatest risk is for susceptible birds that may be exposed to NDV. If isolates of moderate to high virulence for chickens are used for human cancer therapies, those isolates are probably of greater risk for inadvertent exposure of birds and poultry than they are to the humans handling or being treated with those viruses.

ND (produced by moderate or highly virulent forms of the virus) is considered a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals. Laboratory workers should have no contact with susceptible hosts for five days

after working with the agent. Laboratory and animal studies with low virulence viruses or diagnostic accessions should be handled at BSL-2.

Special Issues

Velogenic strains of NDV are USDA select agents. Possession, transfer and use of this agent requires application of procedures as detailed in 9 CFR Part 121, Agricultural Bioterrorism Protection Act of 2002; Possession, Use and Transfer of Biological Agents and Toxins. All rules concerning the possession, storage, use, and transfer of select agents apply. Please review Appendix F of this document for further instructions regarding select agents. An importation or interstate movement permit for NDV must be obtained from USDA/APHIS/VS.

Peste Des Petits Ruminants Virus (PPRV)

PPRV causes disease variously termed stomatitis pneumoenteritis complex, kata, goat plague and pseudorinderpest. The virus affects sheep and especially goats, and is regarded as the most important disease of goats and possibly sheep in West Africa where they are a major source of animal protein. The disease is reported from sub-Saharan Africa north of the equator, the Arabian Peninsula, the Middle East, and the Indian Subcontinent. The virus has particular affinity for lymphoid tissues and epithelial tissue of the gastrointestinal and respiratory tracts, causing high fever, diphtheritic oral plaques, proliferative lip lesions, diarrhea, dehydration, pneumonia and death. In susceptible populations morbidity is commonly 90% and mortality 50-80%, but can reach 100%.[39]

PPRV is a member of the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Morbillivirus*, and species *peste-des-petits-ruminants virus*. Other important morbilliviruses include measles virus, rinderpest virus and canine distemper virus. As in all morbilliviruses, it is pleomorphic, enveloped, about 150 nm in diameter and contains a single molecule of linear, non-infectious, negative sense ssRNA.[40]

The virus is environmentally fragile and requires close direct contact for transmission. Outbreaks typically occur after animal movement and commingling during seasonal migrations or religious festivals. Sources of virus include tears, nasal discharge, coughed secretions, and all secretions and excretions of incubating and sick animals. There is no carrier state, and animals recovering from natural infection have lifetime immunity.

Laboratory Safety and Containment Recommendations

PPRV is not known to infect humans in either laboratory or field settings. The greatest risk of working with PPRV is the escape of the organism into a

susceptible sheep or goat population, which would necessitate USDA emergency procedures to contain and eradicate the disease.

The virus is considered cause of a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals. Laboratory workers should have no contact with susceptible hosts for five days after working with the agent.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Rinderpest Virus (RPV)

Rinderpest (RP) is a highly contagious viral disease of domestic cattle, buffaloes, sheep, goats and some breeds of pigs and a large variety of wildlife species.[41] It is characterized by fever, oral erosions, diarrhea, lymphoid necrosis and high mortality. The disease is present in the Indian subcontinent, Near East and sub-Saharan Africa including Kenya and Somalia.

RPV is a single stranded RNA virus in the family *Paramyxoviridae*, genus *Morbillivirus*. It is immunologically related to canine distemper virus, human measles virus, peste des petits ruminants virus, and marine mammal morbilliviruses. There is only one serotype of RPV including several strains with a wide range of virulence.[42]

RPV is a relatively fragile virus. The virus is sensitive to sunlight, heat, and most disinfectants. It rapidly inactivates at pH 2 and 12. Optimal pH for survival is 6.5 – 7.0. Glycerol and lipid solvents inactivate this virus.

Spread of RPV is by direct and indirect contact with infected animals. Aerosol transmission is not a significant means of transmission. Incubation period varies with strain of virus, dosage, and route of exposure. Following natural exposure, the incubation period ranges from 3 to 15 days but is usually 4 to 5 days.

Laboratory Safety and Containment Recommendations

There are no reports of RPV being a health hazard to humans. The greatest risk of working with RPV is the escape of the organism into susceptible animal populations, which would necessitate USDA emergency procedures to contain and eradicate the disease.

The virus is considered cause of a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved BSL-3-Ag facility for loosely housed animals. Laboratory workers should have no contact with susceptible hosts for five days after working with the agent.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Sheep and Goat Pox Virus (SGPV)

Sheep and goat pox (SGP) is an acute to chronic disease of sheep and goats characterized by generalized pox lesions throughout the skin and mucous membranes, a persistent fever, lymphadenitis, and often a focal viral pneumonia with lesions distributed uniformly throughout the lungs. Subclinical cases may occur. The virus that causes SGP is a capripoxvirus (SGPV), one of the largest viruses (170 – 260 nm by 300 – 450 nm) in the *Poxviridae* family, genus *Capripoxvirus*. It is closely related to the virus that causes lumpy skin disease. The SGPV is very resistant to physical and chemical agents.[43-45]

SGPV causes clinical disease in sheep and goats. The virus replicates in cattle but does not cause clinical disease. The disease has not been detected in wild ungulate populations. It is endemic in Africa, the Middle East, the Indian subcontinent, and much of Asia.

Contact is the main means of transmission of SGPV. Inhalation of aerosols from acutely affected animals, aerosols generated from dust contaminated from pox scabs in barns and night holding areas, and contact through skin abrasions either by fomites or by direct contact are the natural means of transmitting SGPV. Insect transmission (mechanical) is possible. The virus can cause infection experimentally by intravenous, intradermal, intranasal, or subcutaneous inoculation.

Laboratory Safety and Containment Recommendations

Humans are not susceptible to infection by these poxviruses. The greatest risk of working with these agents is the escape of the organism into susceptible domestic animal populations, which would necessitate USDA emergency procedures to contain and eradicate the diseases.[46]

These viruses are considered cause of a foreign animal disease in the United States. Due to the highly contagious nature of the agent and the severe economic consequences of disease presence in the United States, this organism should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Spring Viremia of Carp Virus (SVCV)

Spring Viremia of Carp virus (SVCV) is a rhabdovirus in the family *Rhabdoviridae*, genus *Vesiculovirus* that infects a broad range of fish species and causes high mortality in susceptible hosts in cold water. It is a World Organization for Animal Health Office International des Épizooties (OIE) reportable disease. Infections have occurred in common and koi carp (*Cyprinus carpio*), grass carp (*Crenopharyngodon idellus*), silver carp (*Hypophthalmichthys molitix*), bighead (*Aristichthys nobilis*), cruian carp (*Carassius carassius*), goldfish (*C. auratus*), roach (*Rutilus rutilus*), ide (*Leuciscus idus*), tench (*Tinca tinca*) and sheatfish (*Silurus glanis*). Long indigenous to Europe, the Middle East and Asia, the disease was reported recently in South and North America. In the spring of 2002, SVCV was isolated from koi carp farmed in North Carolina. That year the virus was detected in fish in several lakes and rivers in Wisconsin, including the Mississippi River. SVCV causes impairment in salt-water balance in fish resulting in edema and hemorrhages.

Reservoirs of SVCV are infected fish and carriers from either cultured, feral or wild fish populations.[47] Virulent virus is shed via feces, urine, and gill, skin and mucus exudates. Liver, kidney, spleen, gill and brain are the primary organs containing the virus during infection.[48] It is surmised that horizontal transmission occurs when waterborne virus enters through the gills. Vertical transmission may be possible, especially via ovarian fluids. This virus may remain infective for long periods of time in water or mud. Once the virus is established in a pond or farm, it may be difficult to eradicate without destruction of all fish at the farm.[25,28,49]

Laboratory Safety and Containment Recommendations

Human beings are not susceptible to SVCV infection. The greatest risk of working with SVCV is the escape of the organism into a susceptible fish

population, which would necessitate USDA emergency procedures to contain and eradicate the disease.

SVC is considered a reportable disease in the United States. SVCV should be handled *in vitro* in BSL-2 laboratory facilities with enhancements as required by USDA. Animal inoculations should be handled in ABSL-3 animal facilities with special modifications as required. The OIE Diagnostic Manual for Aquatic Animal Disease has specifications for surveillance programs to achieve and maintain health status of aquaculture facilities.[48] Recommendations for preventing the disease and spread of disease include the use of a water source free of virus, disinfection of eggs and equipment, and proper disposal of dead fish.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

Swine Vesicular Disease Virus (SVDV)

Swine vesicular disease virus (SVDV) is classified in the genus *Enterovirus*, the family *Picornaviridae*, and is closely related to the human enterovirus coxsackievirus B5.[50] The virus is the causative agent of SVD, a contagious disease of pigs characterized by fever and vesicles with subsequent erosion in the mouth and on the snout, feet, and teats.[51,52] The major importance of SVD is that it clinically resembles FMD, and any outbreaks of vesicular disease in pigs must be assumed to be FMD until proven otherwise by laboratory tests.

Occupational Infections

SVDV can cause an "influenza-like" illness in man[1] and human infection has been reported in laboratory personnel working with the virus.[53,54] The virus may be present in blood, vesicular fluid, and tissues of infected pigs. Direct and indirect contacts of infected materials, contaminated laboratory surfaces, and accidental autoinoculation, are the primary hazards to laboratory personnel.

Laboratory Safety and Containment Recommendations

Laboratory practices for SVDV are principally designed to prevent transmission to susceptible livestock, but also to protect workers. Gloves are recommended for the necropsy and handling of infected animals and cell cultures. The greatest risk of working with SVD is the escape of the organism into susceptible animal populations, which would necessitate USDA emergency procedures to contain and eradicate the disease.[55]

SVD is considered a foreign animal disease in the United States. Due to the severe economic consequences of disease presence in the United States, SVDV should only be handled *in vitro* in a BSL-3 laboratory with enhancements as required by the USDA and *in vivo* in a USDA-approved ABSL-3 animal facility with enhancements.

Special Issues

The importation, possession, or use of this agent is prohibited or restricted by law or by USDA regulations or administrative policies. A USDA/APHIS import or interstate movement permit is required to obtain this agent or any livestock or poultry product, such as blood, serum, or other tissues containing the agent.

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VI. Additional Information:

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Veterinary Services, National Center for Import and Export
4700 River Road, Unit 133
Riverdale, Maryland 20737-1231
Telephone: (301) 734-5960
Fax: (301) 734-3256
Internet: *http://www.aphis.usda.gov/animal_health/permits*

Further information on Plant Select Agents, or permits for field release of genetically engineered organisms may be obtained from:

U.S. Department of Agriculture Animal
and Plant Health Inspection Service
Plant Protection and Quarantine, Permits, Agricultural Bioterrorism
4700 River Road, Unit 2
Riverdale, Maryland 20737-1231
Telephone: (301) 734-5960
Internet: *http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml*

Appendix E—Arthropod Containment Guidelines (ACG)

An ad hoc committee of concerned vector biologists including members of the American Committee Medical Entomology (ACME), a subcommittee of the American Society of Tropical Medicine and Hygiene (ASTMH), and other interested persons drafted the "Arthropod Containment Guidelines." The ACG provide principles of risk assessment for arthropods of public health importance. The risk assessment and practices are designed to be consistent with the *NIH Guidelines* for recombinant DNA research and BMBL. Arthropods included are those that transmit pathogens; however, those arthropods that cause myiasis, infestation, biting, and stinging are not included. The ACG also specifically exclude most uses of *Drosophila* spp.

The ACG were published in Vector Borne and Zoonotic Diseases.1 They are freely downloadable from *www.liebertonline.com* and at the AMCE Web site: *www.astmh.org*.

The ACG recommend biosafety measures specific for arthropods of public health importance considering that:

- Arthropods present unique containment challenges not encountered with microbial pathogens.
- Arthropod containment has not been covered specifically in BMBL or the NIH Guidelines.

The ACG contain two sections of greatest interest to most researchers:

1. The Principles of Risk Assessment that discusses arthropods in the usual context (e.g., those known to contain a pathogenic agent, those with uncertain pathogens, and those with no agent).
2. They also consider the following:
   - Biological containment is a significant factor that reduces the hazards associated with accidental escape of arthropods.
   - Epidemiological context alters the risks of an escape and its impact on the location or site in which the work is performed.
   - The phenotype of the vector, such as insecticide resistance; and
   - genetically modified arthropods with an emphasis on phenotypic change.

Four Arthropod Containment Levels (ACL 1 – 4) add increasingly stringent measures and are similar to biosafety levels. The most flexible level is ACL-2 that covers most exotic and transgenic arthropods and those infected with pathogens requiring BSL-2 containment. Like BMBL, each level has the following form:

- standard practices;
- special practices;
- equipment (primary barriers);
- facilities (secondary barriers).

The ACG does not reflect a formal endorsement by ACME or ASTMH. The guidelines are subject to change based on further consideration of the requirements for containment of arthropods and vectors.

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Appendix F—Select Agents and Toxins

The *Public Health Security and Bioterrorism Preparedness and Response Act of 2002, Subtitle A of Public Law 107-188 (42 U.S.C. 262a)*, requires DHHS to regulate the possession, use, and transfer of biological agents or toxins (i.e., select agents and toxins) that could pose a severe threat to public health and safety. The *Agricultural Bioterrorism Protection Act of 2002, Subtitle B of Public Law 107-188 (7 U.S.C. 8401)*, requires the USDA to regulate the possession, use, and transfer of biological agents or toxins (i.e., select agents and toxins) that could pose a severe threat to animal or plant health, or animal or plant products. These Acts require the establishment of a national database of registered entities, and set criminal penalties for failing to comply with the requirements of the Acts. In accordance with these Acts, DHHS and USDA promulgated regulations requiring entities to register with the CDC or the APHIS if they possess, use, or transfer a select agent or toxin (42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121). CDC and APHIS coordinate regulatory activities for those agents that would be regulated by both agencies ("overlap" select agents).

The Attorney General has the authority and responsibility to conduct electronic database checks (i.e., the security risk assessments) on entities that apply to possess, use, or transfer select agents, as well as personnel that require access to select agents and toxins. The FBI, Criminal Justice Information Services Division (CJIS), has been delegated authority for conducting these security risk assessments.

The regulations provide that, unless exempted, entities must register with CDC or APHIS if they possess, use, or transfer select agents or toxins. The current list of select agents and toxins is available on the CDC and APHIS Web sites (see below). The regulations set out a procedure for excluding an attenuated strain of a select agent or toxin and exemptions for certain products and for select agents or toxins identified in specimens presented for diagnosis, verification, or proficiency testing.

The regulations also contain requirements to ensure that the select agents and toxins are handled safely and secured against unauthorized access, theft, loss, or release. For example, entities and their personnel must undergo a security risk assessment by CJIS as part of their registration; entities must limit access to select agents and toxins and develop and implement biosafety, security, and incident response plans. In addition, all select agents or toxins must be transferred in accordance with the regulations and any theft, loss, or release of a select agent or toxin must be reported to CDC or APHIS.

For additional information concerning the select agent regulations, contact CDC or APHIS. Information is also available at the following Web sites: *www.selectagents.gov*; *http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml*.

Appendix G—Integrated Pest Management (IPM)

IPM is an important part of managing a research facility. Many pests, such as flies and cockroaches, can mechanically transmit disease pathogens and compromise the research environment. Even the presence of innocuous insects can contribute to the perception of unsanitary conditions.

The most common approach to pest control has been the application of pesticides, either as a preventive or remedial measure. Pesticides can be effective and may be necessary as a corrective measure, but they have limited long-term effect when used alone. Pesticides also can contaminate the research environment through pesticide drift and volatilization.

To control pests and minimize the use of pesticides, it is necessary to employ a comprehensive program approach that integrates housekeeping, maintenance, and pest control services. This method of pest control is often referred to as IPM. The primary goal of an IPM program is to prevent pest problems by managing the facility environment to make it less conducive to pest infestation. Along with limited applications of pesticides, pest control is achieved through proactive operational and administrative intervention strategies to correct conditions that foster pest problems.

Prior to developing any type of IPM program, it is important to define an operational framework for IPM services that helps promote collaboration between IPM specialists and facility personnel. This framework should incorporate facility restrictions and operational and procedural issues into the IPM program. An effective IPM program is an integral part of the facility's management. An IPM policy statement should be included in the facility's standard operating procedures to increase awareness of the program.

Training sources for the principles and practices of structural (indoor) IPM programs are available through university entomology departments, county extension offices, the Entomological Society of America, state departments of agriculture, state pest control associations, the National Pest Control Association, suppliers of pest control equipment, and IPM consultants and firms. Several universities offer correspondence courses, short courses, and training conferences on structural pest management.

IPM is a strategy-based service that considers not only the cost of the services, but also the effectiveness of the program's components. Each IPM program is site-specific, tailored to the environment where applied.

Laboratory IPM services will be different from those in an office building or an animal care facility. Interrelated components of "Environmental pest management" follow.

Facility Design

IPM issues and requirements should be addressed in a research facility's planning, design, and construction. This provides an opportunity to incorporate features that help exclude pests, minimize pest habitat, and promote proper sanitation in order to reduce future corrections that can disrupt research operations.

Monitoring

Monitoring is the central activity of an IPM program and is used to minimize pesticide use. Traps, visual inspections, and staff interviews identify areas and conditions that may foster pest activity.

Sanitation and Facility Maintenance

Many pest problems can be prevented or corrected by ensuring proper sanitation, reducing clutter and pest habitat, and by performing repairs that exclude pests. Records of structural deficiencies and housekeeping conditions should be maintained to track problems and determine if corrective actions were completed and in a timely manner.

Communication

A staff member should be designated to meet with IPM personnel to assist in resolving facility issues that impact on pest management. Reports communicated verbally and in writing concerning pest activity and improvement recommendations for personnel, practices and facility conditions should be provided to the designated personnel. Facility personnel should receive training on pest identification, biology, and sanitation, which can promote understanding and cooperation with the goals of the IPM program.

Recordkeeping

A logbook should be used to record pest activity and conditions pertinent to the IPM program. It may contain protocols and procedures for IPM services in that facility, Material Safety Data Sheets on pesticides, pesticide labels, treatment records, floor plans, survey reports, etc.

Non-pesticide Pest Control

Pest control methods such as trapping, exclusion, caulking, washing, and freezing can be applied safely and effectively when used in conjunction with proper sanitation and structural repair.

Pest Control with Pesticides

Preventive applications of pesticides should be discouraged, and treatments should be restricted to areas of known pest activity. When pesticides are applied, the least toxic product(s) available should be used and applied in the most effective and safe manner.

Program Evaluation and Quality Assurance

Quality assurance and program review should be performed to provide an objective, ongoing evaluation of IPM activities and effectiveness to ensure that the program does, in fact, control pests and meet the specific needs of the facility program(s) and its occupants. Based upon this review, current IPM protocols can be modified and new procedures implemented.

Technical Expertise

A qualified entomologist can provide helpful technical guidance to develop and implement an IPM program. Pest management personnel should be licensed and certified by the appropriate regulatory agency.

Safety

IPM minimizes the potential of pesticide exposure to the research environment and the staff by limiting the scope of pesticide treatments.

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Appendix H—Working with Human, NHP and Other Mammalian Cells and Tissues

Although risk of laboratory infection from working with cell cultures in general is low, risk increases when working with human and other primate cells, and primary cells from other mammalian species. There are reports of infection of laboratory workers handling primary rhesus monkey kidney cells,[1] and the bloodborne pathogen risks from working with primary human cells, tissues and body fluids are widely recognized.[2,3] OSHA has developed a bloodborne pathogens standard that should be applied to all work in the laboratory with human blood, tissues, body fluids and primary cell lines.[4] Procedures have also been published to reduce contamination of cell cultures with microorganisms.[5,6]

Potential Laboratory Hazards

Potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV, HIV, HCV, HTLV, EBV, HPV and CMV as well as agents such as *Mycobacterium tuberculosis* that may be present in human lung tissue. Other primate cells and tissues also present risks to laboratory workers.[7] Cells immortalized with viral agents such as SV-40, EBV adenovirus or HPV, as well as cells carrying viral genomic material also present potential hazards to laboratory workers. Tumorigenic human cells also are potential hazards as a result of self-inoculation.[8] There has been one reported case of development of a tumor from an accidental needle-stick.[9] Laboratory workers should never handle autologous cells or tissues.[1] NHP cells, blood, lymphoid and neural tissues should always be considered potentially hazardous.

Recommended Practices

Each institution should conduct a risk assessment based on the origin of the cells or tissues (species and tissue type), as well as the source (recently isolated or well-characterized). Human and other primate cells should be handled using BSL-2 practices and containment. All work should be performed in a BSC, and all material decontaminated by autoclaving or disinfection before discarding.[6,10,11,12] BSL-2 recommendations for personnel protective equipment such as laboratory coats, gloves and eye protection should be rigorously followed. All laboratory staff working with human cells and tissues should be enrolled in an occupational medicine program specific for bloodborne pathogens and should work under the policies and guidelines established by the institution's Exposure Control Plan.[4] Laboratory staff working with human cells and tissues should provide a baseline serum sample, be offered hepatitis B immunization, and be evaluated by a health care professional following an exposure incident. Similar programs should be considered for work with NHP blood, body fluids, and other tissues.

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Appendix I—Guidelines for Work with Toxins of Biological Origin

Biological toxins comprise a broad range of poisons, predominantly of natural origin but increasingly accessible by modern synthetic methods, which may cause death or severe incapacitation at relatively low exposure levels.[1,2] Laboratory safety principles are summarized herein for several toxins currently regulated as "Select Agent Toxins," including BoNT, SE, ricin and selected LMW toxins. Additional details are provided in the agent summary statements.

General Considerations for Toxin Use

Laboratory work with most toxins, in amounts routinely employed in the biomedical sciences, can be performed safely with minimal risk to the worker and negligible risk to the surrounding community. Toxins do not replicate, are not infectious, and are difficult to transmit mechanically or manually from person to person. Many commonly employed toxins have very low volatility and, especially in the case of protein toxins, are relatively unstable in the environment; these characteristics further limit the spread of toxins.

Toxins can be handled using established general guidelines for toxic or highly-toxic chemicals with the incorporation of additional safety and security measures based upon a risk assessment for each specific laboratory operation.[3,4] The main laboratory risks are accidental exposure by direct contamination of mouth, eyes or other mucous membranes; by inadvertent aerosol generation; and by needle-sticks or other accidents that may compromise the normal barrier of the skin.

Training and Laboratory Planning

Each laboratory worker must be trained in the theory and practice of the toxins to be used, with special emphasis on the nature of the practical hazards associated with laboratory operations. This includes how to handle transfers of liquids containing toxin, where to place waste solutions and contaminated materials or equipment, and how to decontaminate work areas after routine operations, as well as after accidental spills. The worker must be reliable and sufficiently adept at all required manipulations before being provided with toxin.

A risk assessment should be conducted to develop safe operating procedures before undertaking laboratory operations with toxins; suggested "pre-operational checklists" for working with toxins are available.[4] For complex operations, it is recommended that new workers undergo supervised practice runs in which the exact laboratory procedures to be undertaken are rehearsed without active toxin. If toxins and infectious agents are used together, then both must be considered when containment equipment is selected and safety procedures are developed. Likewise, animal safety practices must be considered for toxin work involving animals.

Each laboratory that uses toxins should develop a specific chemical hygiene plan. The National Research Council has provided a review of prudent laboratory practices when handling toxic and highly toxic chemicals, including the development of chemical hygiene plans and guidelines for compliance with regulations governing occupational safety and health, hazard communication, and environmental protection.[5]

An inventory control system should be in place to account for toxin use and disposition. If toxins are stored in the laboratory, containers should be sealed, labeled, and secured to ensure restricted access; refrigerators and other storage containers should be clearly labeled and provide contact information for trained, responsible laboratory staff.

Laboratory work with toxins should be done only in designated rooms with controlled access and at pre-determined bench areas. When toxins are in use, the room should be clearly posted: "Toxins in Use—Authorized Personnel Only." Unrelated and nonessential work should be restricted from areas where stock solutions of toxin or organisms producing toxin are used. Visitors or other untrained personnel granted laboratory access must be monitored and protected from inadvertently handling laboratory equipment used to manipulate the toxin or organism.

Safety Equipment and Containment

Routine operations with dilute toxin solutions are conducted under BSL-2 conditions with the aid of personal protective equipment and a well-maintained BSC or comparable engineering controls.[6] Engineering controls should be selected according to the risk assessment for each specific toxin operation. A certified BSC or chemical fume hood will suffice for routine operations with most protein toxins. Low molecular weight toxin solutions, or work involving volatile chemicals or radionucleotides combined with toxin solutions, may require the use of a charcoal-based hood filter in addition to HEPA filtration.

All work with toxins should be conducted within the operationally effective zone of the hood or BSC, and each user should verify the inward airflow before initiating work. When using an open-fronted fume hood or BSC, workers should wear suitable laboratory PPE to protect the hands and arms, such as laboratory coats, smocks, or coveralls and disposable gloves. When working with toxins that pose direct percutaneous hazards, special care must be taken to select gloves that are impervious to the toxin and the diluents or solvents employed. When conducting liquid transfers and other operations that pose a potential splash or droplet hazard in an open-fronted hood or BSC, workers should wear safety glasses and disposable facemask, or a face shield.

Toxin should be removed from the hood or BSC only after the exterior of the closed primary container has been decontaminated and placed in a clean secondary container. Toxin solutions, especially concentrated stock solutions, should be transported in leak/spill-proof secondary containers. The interior of the hood or BSC should be decontaminated periodically, for example, at the end of a series of related experiments. Until thoroughly decontaminated, the hood or BSC should be posted to indicate that toxins remain in use, and access should remain restricted.

Selected operations with toxins may require modified BSL-3 practices and procedures**.** The determination to use BSL-3 is made in consultation with available safety staff and is based upon a risk assessment that considers the variables of each specific laboratory operation, especially the toxin under study, the physical state of the toxin (solution or dry form), the total amount of toxin used relative to the estimated human lethal dose, the volume of the material manipulated, the methodology, and any human or equipment performance limitations.

Inadvertent Toxin Aerosols

Emphasis must be placed on evaluating and modifying experimental procedures to eliminate the possibility of inadvertent generation of toxin aerosols. Pressurized tubes or other containers holding toxins should be opened in a BSC, chemical fume hood, or other ventilated enclosure. Operations that expose toxin solutions to vacuum or pressure, for example sterilization of toxin solutions by membrane filtration, should always be handled in this manner, and the operator should also use appropriate respiratory protection. If vacuum lines are used with toxin, they should be protected with a HEPA filter to prevent entry of toxins into the line.

Centrifugation of cultures or materials potentially containing toxins should only be performed using sealed, thick-walled tubes in safety centrifuge cups or sealed rotors. The outside surfaces of containers and rotors should be routinely cleaned before each use to prevent contamination that may generate an aerosol. After centrifugation, the entire rotor assembly is taken from the centrifuge to a BSC to open it and remove its tubes.

Mechanical Injuries

Accidental needle-sticks or mechanical injury from “sharps” such as glass or metal implements pose a well-known risk to laboratory workers, and the consequences may be catastrophic for operations involving toxins in amounts that exceed a human lethal dose.

Only workers trained and experienced in handling animals should be permitted to conduct operations involving injection of toxin solutions using hollow-bore needles. Discarded needles/syringes and other sharps should be placed directly into properly labeled, puncture-resistant sharps containers, and decontaminated as soon as is practical.

Glassware should be replaced with plastic for handling toxin solutions wherever practical to minimize the risk of cuts or abrasions from contaminated surfaces. Thin-walled glass equipment should be completely avoided. Glass Pasteur pipettes are particularly dangerous for transferring toxin solutions and should be replaced with disposable plastic pipettes. Glass chromatography columns under pressure must be enclosed within a plastic water jacket or other secondary container.

Additional Precautions

Experiments should be planned to eliminate or minimize work with dry toxin (e.g., freeze-dried preparations). Unavoidable operations with dry toxin should only be undertaken with appropriate respiratory protection and engineering controls. Dry toxin can be manipulated using a Class III BSC, or with the use of secondary containment such as a disposable glove bag or glove box within a hood or Class II BSC. "Static-free" disposable gloves should be worn when working with dry forms of toxins that are subject to spread by electrostatic dispersal.

In specialized laboratories, the intentional, controlled generation of aerosols from toxin solutions may be undertaken to test antidotes or vaccines in experimental animals. These are extremely hazardous operations that should only be conducted after extensive validation of equipment and personnel, using non-toxic simulants. Aerosol exposure of animals should be done in a certified Class III BSC or hoodline. While removing exposed animals from the hoodline, and for required animal handling during the first 24 h after exposure, workers should take additional precautions, including wearing protective clothing (e.g., disposable Tyvek suit) and appropriate respiratory protection. To minimize the risk of dry toxin generating a secondary aerosol, areas of animal skin or fur exposed to aerosols should be gently wiped with a damp cloth containing water or buffered cleaning solution before the animals are returned to holding areas.

For high-risk operations involving dry forms of toxins, intentional aerosol formation, or the use of hollow-bore needles in conjunction with amounts of toxin estimated to be lethal for humans, consideration should be given to requiring the presence of at least two knowledgeable individuals at all times in the laboratory.[7]

Decontamination and Spills

Toxin stability varies considerably outside of physiological conditions depending upon the temperature, pH, ionic strength, availability of co-factors and other characteristics of the surrounding matrix. Literature values for dry heat inactivation of toxins can be misleading due to variations in experimental conditions, matrix composition, and experimental criteria for assessing toxin activity. Moreover, inactivation is not always a linear function of heating time; some protein toxins possess a capacity to re-fold and partially reverse

inactivation caused by heating. In addition, the conditions for denaturizing toxins in aqueous solutions are not necessarily applicable for inactivating dry, powdered toxin preparations.

General guidelines for laboratory decontamination of selected toxins are summarized in Tables 1 and 2, but inactivation procedures should not be assumed to be 100% effective without validation using specific toxin bioassays. Many toxins are susceptible to inactivation with dilute sodium hydroxide (NaOH) at concentrations of 0.1-0.25N, and/or sodium hypochlorite (NaOCl) bleach solutions at concentrations of 0.1-0.5% (w/v). Use freshly prepared bleach solutions for decontamination; undiluted, commercially available bleach solutions typically contain 3-6% (w/v) NaOCl.

Depending upon the toxin, contaminated materials and toxin waste solutions can be inactivated by incineration or extensive autoclaving, or by soaking in suitable decontamination solutions (Table 2). All disposable material used for toxin work should be placed in secondary containers, autoclaved and disposed of as toxic waste. Contaminated or potentially contaminated protective clothing and equipment should be decontaminated using suitable chemical methods or autoclaving before removal from the laboratory for disposal, cleaning or repair. If decontamination is impracticable, materials should be disposed of as toxic waste.

In the event of a spill, avoid splashes or generating aerosols during cleanup by covering the spill with paper towels or other disposable, absorbent material. Apply an appropriate decontamination solution to the spill, beginning at the perimeter and working towards the center, and allow sufficient contact time to completely inactivate the toxin (Table 2).

Decontamination of buildings or offices containing sensitive equipment or documents poses special challenges. Large-scale decontamination is not covered explicitly here, but careful extrapolation from the basic principles may inform more extensive clean-up efforts.

Select Agent Toxins

Due diligence should be taken in shipment or storage of any amount of toxin. There are specific regulatory requirements for working with toxins designated as a “Select Agent” by the DHHS and/or the USDA. Select agents require registration with CDC and/or USDA for possession, use, storage and/or transfer. Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of the agent may require a permit from USDA/ APHIS/VS. A DoC permit may be required for the export of the agent to another country. See Appendix C for additional information.

Table 1. Physical Inactivation of Selected Toxins

Toxin	Steam Autoclave	Dry Heat (10 min)	Freeze-thaw	Gamma Irradiation
Botulinum neurotoxin	Yes [a]	> 100º C [b]	No [c]	Incomplete [d]
Staphylococcal Enterotoxin	Yes [e]	> 100º C; refolds [f]	No [g]	Incomplete [h]
Ricin	Yes [i]	> 100º C [i]	No [j]	Incomplete [k]
Microcystin	No [l]	> 260º C [m]	No [n]	ND
Saxitoxin	No [l]	> 260º C [m]	No [n]	ND
Palytoxin	No [l]	> 260º C [m]	No [n]	ND
Tetrodotoxin	No [l]	> 260º C [m]	No [n]	ND
T-2 mycotoxin	No [l]	> 815º C [m]	No [n]	ND
Brevetoxin (PbTx-2)	No [l]	> 815º C [m]	No [n]	ND

Notes:

ND indicates “not determined” from available decontamination literature.

[a] Steam autoclaving should be at >121°C for 1 h. For volumes larger than 1 liter, especially those containing *Clostridium botulinum* spores, autoclave at >121°C for 2 h to ensure that sufficient heat has penetrated to kill all spores.[8,9]

[b] Exposure to 100°C for 10 min. inactivates BoNT. Heat denaturation of BoNT as a function of time is biphasic with most of the activity destroyed relatively rapidly, but with some residual toxin (e.g., 1-5%) inactivated much more slowly.[10]

[c] Measured using BoNT serotype A at -20°C in food matrices at pH 4.1 – 6.2 over a period of 180 days.[11]

[d] Measured using BoNT serotypes A and B with gamma irradiation from a ^{60}Co source.[12,13]

[e] Protracted steam autoclaving, similar to that described for BoNT, followed by incineration is recommended for disposal of SE-contaminated materials.

[f] Inactivation may not be complete depending upon the extent of toxin re-folding after denaturation. Biological activity of SE can be retained despite heat and pressure treatment routinely used in canned food product processing.[14]

[g] SE toxins are resistant to degradation from freezing, chilling or storage at ambient temperature.[15] Active SEB in the freeze-dried state can be stored for years.

[h] References [15,16]

[i] Dry heat of >100ºC for 60 min in an ashing oven or steam autoclave treatment at >121ºC for 1 h reduced the activity of pure ricin by >99%.[17] Heat inactivation of impure toxin preparations (e.g., crude ricin plant extracts) may vary. Heat-denatured ricin can undergo limited refolding (<1%) to yield active toxin.

[j] Ricin holotoxin is not inactivated significantly by freezing, chilling or storage at ambient temperature. In the liquid state with a preservative (sodium azide), ricin can be stored at 4ºC for years with little loss in potency.

[k] Irradiation causes a dose-dependent loss of activity for aqueous solutions of ricin, but complete inactivation is difficult to achieve; 75 MRad reduced activity 90%, but complete inactivation was not achieved even at 100 MRad.[18] Gamma irradiation from a laboratory ^{60}Co source can be used to partially inactivate aqueous solutions of ricin, but dried ricin powders are significantly resistant to inactivation by this method.

[l] Autoclaving with 17 lb pressure (121-132º C) for 30 min failed to inactivate LMW toxins.[17,19] All burnable waste from LMW toxins should be incinerated at temperatures in excess of 815°C (1,500º F).

[m] Toxin solutions were dried at 150º C in a crucible, placed in an ashing oven at various temperatures for either 10 or 30 min, reconstituted and tested for concentration and/or activity; tabulated values are temperatures exceeding those required to achieve 99% toxin inactivation.[17]

[n] LMW toxins are generally very resistant to temperature fluctuations and can be stored in the freeze-dried state for years and retain toxicity.

Table 2. Chemical Inactivation of Selected Toxins

Toxin	NaOCl (30 min)	NaOH (30 min)	NaCOl + NaOH (30 min)	Ozone Treatment
Botulinum neurotoxin	> 0.1% [a]	> 0/25 N	ND	Yes [b]
Staphylococcal Enterotoxin	> 0.5% [c]	> 0.25 N	ND	ND
Ricin	> 1.0% [d]	ND	> 0.1% + 0.25N [e]	ND
Saxitoxin	≥ 0.1% [e]	ND	0.25% + 0.25N [e]	ND
Palytoxin	≥ 0.1% [e]	ND	0.25% + 0.25N [e]	ND
Microcystin	≥ 0.5% [e]	ND	0.25% + 0.25N [e]	ND
Tetrodotoxin	≥ 0.5% [e]	ND	0.25% + 0.25N [e]	ND
T-2 mycotoxin	≥ 2.5% [e, f]	ND	0.25% + 0.25N [e]	ND
Brevetoxin (PbTx-2)	≥ 2.5% [e, f]	ND	0.25% + 0.25N [e]	ND

Notes:
ND indicates "not determined" from available decontamination literature.

[a] Solutions of NaOCl (#0.1%) or NaOH (> 0.25 N) for 30 min inactivate BoNT and are recommended for decontaminating work surfaces and spills of *C. botulinum* or BoNT. Chlorine at a concentration of 0.3-0.5 mg/L as a solution of hypochlorite rapidly inactivates BoNT (serotypes B or E tested) in water.[20] Chlorine dioxide inactivates BoNT, but chloramine is less effective.[21]

[b] Ozone (> 2 mg/L) or powdered activated charcoal treatment also completely inactivate BoNT (serotypes A, B tested) in water under defined condition.[20,22]

[c] SEB is inactivated with 0.5% hypochlorite for 10-15 mi.[23]

[d] Ricin is inactivated by a 30 min exposure to concentrations of NaOCl ranging from 0.1-2.5%, or by a mixture of 0.25% NaOCl plus 0.25 N NaOH.[17] In general, solutions of 1.0% NaOCl are effective for decontamination of ricin from laboratory surfaces, equipment, animal cages, or small spills.

[e] The minimal effective concentration of NaOCl was dependent on toxin and contact time; all LMW toxins tested were inactivated at least 99% by treatment with 2.5% NaOCl, or with a combination of 0.25% NaOCl and 0.25N NaOH.[17]

[f] For T-2 mycotoxin and brevetoxin, liquid samples, accidental spills, and nonburnable waste should be soaked in 2.5% NaOCl with 0.25% N NaOH for 4 h. Cages and bedding from animals exposed to T-2 mycotoxin or brevetoxin should be treated with 0.25% NaOCl and 0.025 N NaOH for 4 h. Exposure for 30 min to 1.0% NaOCl is an effective procedure for the laboratory (working solutions, equipment, animal cages, working area and spills) for the inactivation of saxitoxin or tetrodotoxin. Decontamination of equipment and waste contaminated with select brevetoxins has been reviewed.[19]

Alternate methods of chemical decontamination: 1 N sulfuric or hydrochloric acid did not inactivate T-2 mycotoxin and only partially inactivated microcystin-LR, saxitoxin, and brevetoxin (PbTx-2). Tetrodotoxin and palytoxin were inactivated by hydrochloric acid, but only at relatively high molar concentrations. T2 was not inactivated by exposure to 18% formaldehyde plus methanol (16 h), 90% freon-113 + 10% acetic acid, calcium hypochlorite, sodium bisulfate, or mild oxidizing.17 Hydrogen peroxide was ineffective in inactivating T-2 mycotoxin. This agent did cause some inactivation of saxitoxin and tetrodotoxin, but required a 16 h contact time in the presence of ultraviolet light.

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Appendix J—NIH Oversight of Research Involving Recombinant Biosafety Issues

The NIH locus for oversight of recombinant DNA research is the Office of Biotechnology Activities (OBA), which is located within the Office of Science Policy, in the Office of the Director of the NIH. The OBA implements and manages the various oversight tools and information resources that NIH uses to promote the science, safety and ethics of recombinant DNA research. The key tools of biosafety oversight are the *NIH Guidelines*, IBCs, and the Recombinant DNA Advisory Committee (RAC). The NIH also undertakes special initiatives to promote the analysis and dissemination of information key to our understanding of recombinant DNA, including human gene transfer research. These initiatives include a query-capable database and conferences and symposia on timely scientific, safety, and policy issues. The NIH system of oversight is predicated on ethical and scientific responsibilities, with goals to promote the exchange of important scientific information, enable high-quality research, and help advance all fields of science employing recombinant DNA.

The *NIH Guidelines* promote safe conduct of research involving recombinant DNA by specifying appropriate biosafety practices and procedures for research involving the construction and handling of either recombinant DNA molecules or organisms and viruses that contain recombinant DNA. Recombinant DNA molecules are defined in the *NIH Guidelines* as those constructed outside of a living cell by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell. The *NIH Guidelines* are applicable to all recombinant DNA work at an institution that receives any funding from the NIH for recombinant DNA research. Compliance with the *NIH Guidelines* is mandatory for investigators conducting recombinant DNA research funded by the NIH or performed at or sponsored by any public or private entity that receives any NIH funding for recombinant DNA research. This broad reach of the *NIH Guidelines* is intended to instill biosafety practices throughout the institution, which is necessary if the practices are to be effective.

The *NIH Guidelines* were first published in 1976 and are revised as technological, scientific, and policy developments warrant. They outline the roles and responsibilities of various entities associated with recombinant DNA research, including institutions, investigators, biological safety officers, and the NIH (see Section IV of the *NIH Guidelines*). They describe four levels of biosafety and containment practices that correspond to the potential risk of experimentation and require different levels of review for recombinant DNA research, based on the nature and risks of the activity. These include:

1. Review by the RAC, and approval by the NIH Director and the IBC.

2. Review by the NIH OBA and approval by the IBC.

3. Review by the RAC and approvals by the IBC and Institutional Review Board.

4. Approval by the IBC prior to initiation of the research.

5. Notification of the IBC simultaneous with initiation of the work.

See Section III of the *NIH Guidelines* for additional details. In all instances, it is important to note that review by an IBC is required.

The federally mandated responsibilities for an IBC are articulated solely in the *NIH Guidelines*. Their membership, procedures, and functions are outlined in Section IV-B-2. Institutions, ultimately responsible for the effectiveness of IBCs, may define additional roles and responsibilities for these committees in addition to those specified in the Guidelines. To access the NIH Guidelines see the following Web site: *http://oba.od.nih.gov/rdna/nih_guidelines_oba.html*.

The Recombinant DNA Advisory Committee is a panel of national experts in various fields of science, medicine, genetics, and ethics. It includes individuals who represent patient perspectives. The RAC considers the current state of knowledge and technology regarding recombinant DNA research and advises the NIH Director and OBA on basic and clinical research involving recombinant DNA and on the need for changes to the *NIH Guidelines*.

Additional information on OBA, the *NIH Guidelines*, and the NIH RAC can be found at: *http://oba.od.nih.gov*.

Appendix K—Resources

Resources for information, consultation, and advice on biohazard control, decontamination procedures, and other aspects of laboratory and animal safety management include:

AAALAC International
Association for Assessment and Accreditation
of Laboratory Animal Care International
5283 Corporate Drive
Suite 203
Fredrick, MD 21703-2879
Telephone: (301) 696-9626
Fax: (301) 696-9627
Web site: *http://www.aaalac.org*

American Biological Safety Association (ABSA)
American Biological Safety Association
1200 Allanson Road
Mundelein, IL 60060-3808
Telephone: (847) 949-1517
Fax: (847) 566-4580
Web site: *http://www.absa.org*

CDC Etiologic Agent Import Permit Program
Centers for Disease Control and Prevention
Etiologic Agent Import Permit Program
1600 Clifton Road, NE Mailstop: F-46
Atlanta, GA 30333
Telephone: (404) 718-2077
Fax: (404) 718-2093
Web site: *http://www.cdc.gov/od/eaipp*

CDC Office of Health and Safety
Centers for Disease Control and Prevention
Office of Health and Safety
Mailstop: F-05
1600 Clifton Road, NE
Atlanta, GA 30333
Telephone: (404) 639-7233
Fax: (404) 639-2294
Web site: *www.cdc.gov/biosafety*

CDC Select Agent Program
Centers for Disease Control and Prevention
Division of Select Agents and Toxins
Mailstop: A-46
1600 Clifton Road, NE
Atlanta, GA 30333
Telephone: (404) 718-2000
Fax: (404) 718-2096
Web site: *www.selectagents.gov*

Clinical and Laboratory Standards Institute
940 West Valley Road, Suite 1400
Wayne, PA 19087
Telephone: (610) 688-0100
Fax: (610) 688-0700
Web site: *http://www.clsi.org*

College of American Pathologists
1350 I St. N.W. Suite 590
Washington, DC 20005-3305
Telephone: (800) 392-9994
Telephone: (202) 354-7100
Fax: (202) 354-7155
Web site: *http://www.cap.org*

Department of the Army
Biological Defense Safety Program
Department of Defense
32 CFR Parts 626, 627
Web site: *www.gpo.gov*

National B-Virus Resource Laboratory
National B Virus Resource Laboratory
Attention: Dr. Julia Hillard
Viral Immunology Center
Georgia State University
50 Decatur Street
Atlanta, GA 30303
Telephone: (404) 651-0808
Fax: (404) 651-0814
Web site: *http://www2.gsu.edu/~wwwvir*

NIH Division of Occupational Health and Safety
National Institutes of Health
Division of Occupational Health and Safety
Building 13, Room 3K04
13 South Drive, MSC 5760
Bethesda, MD 20892
Telephone: (301) 496-2960
Fax: (301) 402-0313
Web site: *http://dohs.ors.od.nih.gov/index.htm*

NIH Office of Biotechnology Activities
National Institutes of Health
Office of Biotechnology Activities
6705 Rockledge Drive
Suite 750, MSC 7985
Bethesda, MD 20892
Telephone: (301) 496-9838
Fax: (301) 496-9839
Web site: *http://oba.od.nih.gov*

NIH Office of Laboratory Animal Welfare (OLAW)
National Institutes of Health
Office of Laboratory Animal Welfare (OLAW)
RKL 1, Suite 360, MSC 7982
6705 Rockledge Drive
Bethesda, MD 20892-7982
Telephone: (301) 496-7163
Web site: *http://grants.nih.gov/grants/olaw/olaw.htm*

Occupational Safety and Health Administration
U.S. Department of Labor
200 Constitution Avenue NW
Washington, DC 20210
Telephone: (800) 321-6742
Web site: *http://www.osha.gov/index.html*

USDA-APHIS National Center for Import / Export
USDA Animal and Plant Health Inspection Service Veterinary Services National Center for Import and Export
4700 River Road, Unit 40
Riverdale, MD 20737
Web site: *http://www.aphis.usda.gov/import_export/index.shtml*

USDA Agriculture Select Agent Program
USDA Agriculture Select Agent Program
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Road, Unit 2, Mailstop 22
Riverdale, MD 20737
Web site: *http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml*

USDA National Animal Disease Center
U.S. Department of Agriculture
National Animal Disease Center
P.O. Box 70
2300 Dayton Road
Ames, IA 50010
Telephone: (515) 663-7200
Fax: (515) 663-7458
Web site: *http://www.ars.usda.gov/main/site_main.htm?modecode=36-25-30-00*

USDA Plant Select Agents & Plant Protection and Quarantine
Animal and Plant Health Inspection Service
Plant Protection and Quarantine, Permits, Agricultural Bioterrorism
U.S. Department of Agriculture
4700 River Road, Unit 133
Riverdale, MD 20737
Telephone: (877) 770-5990
Fax: (301) 734-5786
Web site: *http://www.aphis.usda.gov/programs/ag_selectagent/index.shtml*;
and *http://www.aphis.usda.gov/permits/index.shtml*

US Department of Transportation
Hazardous Materials Center
Pipeline & Hazardous Materials Center
U.S. Department of Transportation
400 7th Street, S.W.
Washington, DC 20590
Telephone: (800) 467-4922
Web site: *http://www.phmsa.dot.gov/hazmat*

US Department of Commerce
Export Administration Program
Bureau of Industry and Security (BIS)
Export Administration Regulations (EAR)
U.S. Department of Commerce
14th Street and Constitution Avenue, N.W.
Washington, DC 20230
Telephone: (202) 482-4811
Web site: *http://www.access.gpo.gov/bis/index.html*

World Health Organization Biosafety Program
World Health Organization Biosafety Program
Avenue Appia 20
1211 Geneva 27
Switzerland
Telephone: (+ 41 22) 791 21 11
Fax: (+ 41 22) 791 3111
Web site: *http://www.who.int/ihr/biosafety/en*

Appendix L—Acronyms

A1HV-1	Alcelaphine Herpesvirus-1
ABSA	American Biological Safety Association
ABSL	Animal Biosafety Level
ACAV	American Committee on Arthropod-Borne Viruses
ACIP	Advisory Committee on Immunization Practices
ACG	Arthropod Containment Guidelines
ACL	Arthropod Containment Levels
ACME	American Committee of Medical Entomology
AHS	African Horse Sickness
AHSV	African Horse Sickness Virus
AKAV	Akabane Virus
APHIS	Animal and Plant Health Inspection Service
APMV-1	Avian Paramyxovirus Type 1
ASF	African Swine Fever
ASFV	African Swine Fever Virus
ASHRAE	American Society of Heating, Refrigerating, and Air-Conditioning Engineers
ASTMH	American Society of Tropical Medicine and Hygiene
BCG	Bacillus Calmette-Guérin
BDV	Border Disease Virus
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BoNT	Botulinium neurotoxin
BSC	Biological Safety Cabinet
BSE	Bovine Spongiform Encephalopathy
BSL	Biosafety Level
BSL-3-Ag	BSL-3-Agriculture
BSO	Biological Safety Officer
BTV	Bluetongue Virus
BVDL	Bovine Viral Diarrhea Virus
CAV	Constant Air Volume
CBPP	Contagious Bovine Pleuropneumonia
CCPP	Contagious Caprine Pleuropneumonia
CETBE	Central European Tick-Borne Encephalitis
CDC	Centers for Disease Control and Prevention
CHV-1	Cercopithecine Herpesvirus-1
CJD	Creutzfeldt-Jakob Disease
CJIS	Criminal Justice Information Services Division
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CSFV	Classical Swine Fever Virus
DHHS	Department of Health and Human Services
DoC	Department of Commerce
DOD	Department of Defense
DOL	Department of Labor

DOT	Department of Transportation
EBV	Epstein-Barr Virus
EEE	Eastern Equine Encephalomyelitis
EPA	Environmental Protection Agency
EtOH	Ethanol
FDA	Food and Drug Administration
FFI	Fatal Familial Insomnia
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GI	Gastrointestinal Tract
GSS	Gerstmann-Straussler-Scheinker Syndrome
HEPA	High Efficiency Particulate Air
HBV	Hepatitis B Virus
HCMV	Human Cytomegalovirus
HCV	Hepatitis C Virus
HD	Heartwater Disease
HDV	Hepatitis D Virus
HFRS	Hemorrhagic Fever with Renal Syndrome
HHV	Human Herpes Virus
HHV-6A	Human Herpes Virus -6A
HHV-6B	Human Herpes Virus -6B
HHV-7	Human Herpes Virus -7
HHV-8	Human Herpes Virus -8
HIV	Human Immunodeficiency Virus
HPAI	Highly Pathogenic Avian Influenza
HPAIV	Highly Pathogenic Avian Influenza Virus
HPS	Hantavirus Pulmonary Syndrome
HSV-1	Herpes Simplex Virus-1
HSV-2	Herpes Simplex Virus-2
HTLV	Human T-Lymphotropic Viruses
HVAC	Heating, Ventilation, and Air Conditioning
IACUC	Institutional Animal Care and Use Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
ICAO	International Civil Aviation Organization
ID	Infectious Dose
ID_{50}	Number of organisms necessary to infect 50% of a group of animals
IgG	Immunoglobulin
ILAR	Institute for Laboratory Animal Research
IND	Investigational New Drug
IPM	Integrated Pest Management
IPV	Inactivated Poliovirus Vaccine
ISA	Infectious Salmon Anemia

ISAV	Infectious Salmon Anemia Virus
LAI	Laboratory-Associated Infections
LCM	Lymphocytic Choriomeningitis
LCMV	Lymphocytic Choriomeningitis Virus
LD	Lethal Dose
lfm	Linear Feet Per Minute
LGV	Lymphogranuloma Venereum
LMW	Low Molecular Weight
LSD	Lumpy Skin Disease
LSDV	Lumpy Skin Disease Virus
MCF	Malignant Catarrhal Fever
MenV	Menangle Virus
MMWR	Morbidity and Mortality Weekly Report
MPPS	Most Penetrating Particle Size
NaOCl	Sodium Hypochlorite
NaOH	Sodium Hydroxide
NBL	National Biocontainment Laboratory
NCI	National Cancer Institute
ND	Newcastle Disease
NDV	Newcastle Disease Virus
NHP	Nonhuman Primate
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
OBA	NIH Office of Biotechnology Activities
OIE	World Organization for Animal Health
OPV	Oral Poliovirus Vaccine
OSHA	Occupational Safety and Health Administration
PAPR	Positive Air-Purifying Respirator
PBT	Pentavalent Botulinum Toxoid Vaccine
PPD	Purified Protein Derivative
PPM	Parts Per Million
PPRV	Pest des Petits Ruminants Virus
Prp	Prion Protein
RAC	Recombinant DNA Advisory Committee
RBL	Regional Biocontainment Laboratory
RP	Rinderpest
RPV	Rinderpest Virus
RVF	Rift Valley Fever
RVFV	Rift Valley Fever Virus
SALS	Subcommittee on Arbovirus Laboratory Safety
SARS	Severe Acute Respiratory Syndrome
SARS-CoV	SARS-Associated Coronavirus
SCID	Severe Combined Immune Deficient
SC type	Small-Colony type

SE	Staphylococcal Enterotoxins
SEA	SE Serotype A
SEB	SE Serotype B
SIV	Simian Immunodeficiency Virus
SGP	Sheep and Goat Pox
SGPV	Sheep and Goat Pox Virus
SOP	Standard Operating Procedure
SVCV	Spring Viremia of Carp Virus
SVD	Swine Vesicular Disease
SVDV	Swine Vesicular Disease Virus
TLV	Threshold Limit Values
TME	Transmissible Mink Encephalopathy
TSE	Transmissible Spongiform Encephalopathy
UV	Ultraviolet
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases
USDA	U.S. Department of Agriculture
USPS	United States Postal Service
UPS	Uninterrupted Power Supply
VAV	Variable Air Volume
VEE	Venezuelan Equine Encephalitis
VS	Veterinary Services
VZV	Varicella-Zoster Virus
WEE	Western Equine Encephalomyelitis
WHO	World Health Organization
WNV	West Nile Virus

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