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All persons leaving the BSL-4/ABSL-4 laboratory are required to take a personal body shower.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Use of needles and syringes or other sharp instruments are limited for use in the animal facility is limited to situations where there is no alternative such as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
 - b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal and placed as close to the work site as possible.
 - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
 - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
 - e. Equipment containing sharp edges and corners should be avoided.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.

Procedures involving the manipulation of infectious materials must be conducted within biological safety cabinets, or other physical containment devices. When procedures cannot be performed in a BSC, alternate containment equipment should be used.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All incidents must be reported to the animal facility director, laboratory supervisor, institutional management and appropriate facility safety personnel. Medical evaluation, surveillance, and treatment must be provided and appropriate records maintained.

8. Decontaminate all wastes (including animal tissues, carcasses, and contaminated bedding) and other materials before removal from the ABSL-4 laboratory by an effective and validated method. Laboratory clothing should be decontaminated before laundering.

Supplies and materials needed in the facility must be brought in through a double-door autoclave, fumigation chamber, or airlock. Supplies and materials that are not brought into the ABSL-4 laboratory through the change room must be brought in through a previously decontaminated double-door autoclave, fumigation chamber, or airlock. Containment should be maintained at all times. After securing the outer doors, personnel within the areas where infectious materials and/or animals are housed or are manipulated retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors must be secured after materials are brought into the facility.

Only necessary equipment and supplies should be taken inside the ABSL-4 laboratory. All equipment and supplies taken inside the laboratory must be decontaminated before removal. Consideration should be given to means for decontaminating routine husbandry equipment and sensitive electronic and medical equipment.

The doors of the autoclave and fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave has been operated through a decontamination cycle or the fumigation chamber has been decontaminated.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory and the animal room/s when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room.

Security sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy.

Advance consideration must be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.^{1,3,4}

10. An effective integrated pest management program is required.
(See Appendix G.)
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.
12. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or are manipulated.

B. Special Practices

1. All persons entering the ABSL-4 laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

Only persons whose presence in the laboratory or individual animal rooms is required for scientific or support purposes are authorized to enter.

Entry into the facility must be limited by means of secure, locked doors. A logbook, or other means of documenting the date and time of all persons entering and leaving the ABSL-4 laboratory must be maintained.

While the laboratory is operational, personnel must enter and exit the laboratory through the clothing change and shower rooms except during emergencies. All personal clothing must be removed in the outer clothing change room. All personnel entering the laboratory must use laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves.

All persons leaving the ABSL-4 laboratory are required to take a personal body shower. Used laboratory clothing must not be removed

from the inner change room through the personal shower. These items must be treated as contaminated materials and decontaminated before laundering or disposal.

After the laboratory has been completely decontaminated by validated method, necessary staff may enter and exit the laboratory without following the clothing change and shower requirements described above.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

2. Animal facility personnel and support staff must be provided occupational medical services, including medical surveillance and available immunizations for agents handled or potentially present in the laboratory. A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-acquired illnesses. An essential adjunct to an occupational medical system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory-acquired illnesses.
3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
4. The animal facility supervisor is responsible for ensuring that animal personnel:
 - a. Receive appropriate training in the practices and operations specific to the animal facility, such as animal husbandry procedures, potential hazards present, manipulations of infectious agents, and necessary precautions to prevent potential exposures.
 - b. Demonstrate high proficiency in standard and special microbiological practices, and techniques before entering the ABSL-4 facility or working with agents requiring ABSL-4 containment.
 - c. Receive annual updates and additional training when procedure or policy changes occur. Records are maintained for all hazard evaluations and employee training.
5. Removal of biological materials that are to remain in a viable or intact state from the ABSL-4 laboratory must be transferred to a non-breakable,

sealed primary container and then enclosed in a non-breakable, sealed secondary container. These materials must be transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower. Once removed, packaged viable material must not be opened outside ABSL-4 containment unless inactivated by a validated method.

6. Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination. Equipment, cages, and racks should be handled in manner that minimizes contamination of other areas. Cages are autoclaved or thoroughly decontaminated before they are cleaned and washed.
 - a. All equipment and contaminated materials must be decontaminated before removal from the animal facility. Equipment must be decontaminated using an effective and validated method before repair, maintenance, or removal from the animal facility.
 - b. Equipment or material that might be damaged by high temperatures or steam must be decontaminated using an effective and validated procedure such as a gaseous or vapor method in an airlock or chamber designed for this purpose.
 - c. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material. A spill procedure must be developed and posted within the laboratory. Spills and accidents of potentially infectious materials must be immediately reported to the animal facility and laboratory supervisors or personnel designated by the institution.
7. The doors of the autoclave and fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave/ decontamination chamber has been operated through a decontamination cycle or the fumigation chamber has been decontaminated.
8. Daily inspections of essential containment and life support systems must be completed before laboratory work is initiated to ensure that the laboratory and animal facilities are operating according to established parameters.
9. Practical and effective protocols for emergencies must be established. These protocols must include plans for medical emergencies, facility malfunctions, fires, escape of animals within the ABSL-4 laboratory, and other potential emergencies. Training in emergency response procedures must be provided to emergency response personnel according to institutional policies.

10. Based on site-specific risk assessment, personnel assigned to work with infected animals may be required to work in pairs. Procedures to reduce possible worker exposure must be instituted, such as use of squeeze cages, working only with anesthetized animals, or other appropriate practices.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

Cabinet Laboratory

1. All manipulations of infectious animals and materials within the laboratory must be conducted in the Class III BSC. Double-door, pass through autoclaves must be provided for decontaminating materials passing out of the Class III BSC(s). The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

The Class III cabinet must also have a pass-through dunk tank, fumigation chamber, or equivalent decontamination method so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet. Containment must be maintained at all times.

The Class III cabinet must have a HEPA filter on the supply air intake and two HEPA filters in series on the exhaust outlet of the unit. There must be gas-tight dampers on the supply and exhaust ducts of the cabinet to permit gas or vapor decontamination of the unit. Ports for injection of test medium must be present on all HEPA filter housings.

The interior of the Class III cabinet must be constructed with smooth finishes that can be easily cleaned and decontaminated. All sharp edges on cabinet finishes must be eliminated to reduce the potential for cuts and tears of gloves. Equipment to be placed in the Class III cabinet should also be free of sharp edges or other surfaces that may damage or puncture the cabinet gloves.

Class III cabinet gloves must be inspected for leaks periodically and changed if necessary. Gloves should be replaced annually during cabinet re-certification.

The cabinet should be designed to permit maintenance and repairs of cabinet mechanical systems (refrigeration, incubators, centrifuges, etc.) to be performed from the exterior of the cabinet whenever possible.

Manipulation of high concentrations or large volumes of infectious agents within the Class III cabinet should be performed using physical containment devices inside the cabinet whenever practical. Such

materials should be centrifuged inside the cabinet using sealed rotor heads or centrifuge safety cups.

The interior of the Class III cabinet as well as all contaminated plenums, fans and filters must be decontaminated using a validated gaseous or vapor method.

The Class III cabinet must be certified at least annually.

Restraint devices and practices that reduce the risk of exposure during animal manipulations must be used where practicable (e.g., physical restraint devices, chemical restraint medications, mesh or Kevlar gloves, etc.).

2. Workers must wear protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls when in the laboratory. No personal clothing, jewelry, or other items except eyeglasses should be taken past the personal shower area. Upon exiting the laboratory, all protective clothing must be removed in the dirty side change room before showering. Reusable laboratory clothing must be autoclaved before being laundered.
3. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment. Prescription eye glasses must be decontaminated before removal through the personal body shower.
4. Gloves must be worn to protect against breaks or tears in the cabinet gloves. Gloves must not be worn outside the laboratory. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

Suit Laboratory

1. Infected animals should be housed in a primary containment system (such as open cages placed in ventilated enclosures, solid wall and bottom cages covered with filter bonnets and opened in laminar flow hoods, or other equivalent primary containment systems).

Personnel wearing a one-piece positive pressure suit ventilated with a life support system must conduct all procedures.

All manipulations of potentially infectious agents must be performed within a Class II BSC or other primary barrier system. Infected animals should be handled within a primary barrier system, such as a Class II BSC or other equivalent containment system.

Equipment that may produce aerosols must be contained in devices that exhaust air through HEPA filtration before being discharged into the laboratory. These HEPA filters should be tested annually and replaced as need.

HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations.

2. Workers must wear protective laboratory clothing, such as scrub suits, before entering the room used for donning positive pressure suits. All protective clothing must be removed in the dirty side change room before entering the personal shower. Reusable laboratory clothing must be autoclaved before being laundered.
3. Inner gloves must be worn to protect against break or tears in the outer suit gloves. Disposable gloves must not be worn outside the change area. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Inner gloves must be removed and discarded in the inner change room prior to entering the personal shower. Dispose of used gloves with other contaminated waste.
4. Decontamination of outer suit gloves is performed during operations to remove gross contamination and minimize further contamination of the laboratory.

D. Laboratory Facilities (Secondary Barriers)

Cabinet Laboratory

1. The ABSL-4 cabinet laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

Rooms in the ABSL-4 facility must be arranged to ensure sequential passage through an inner (dirty) change area, personal shower and outer (clean) change room prior to exiting the room(s) containing the Class III BSC(s).

An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit, and security systems should be on an uninterrupted power supply (UPS).

A double-door autoclave, dunk tank, fumigation chamber, or ventilated anteroom/airlock must be provided at the containment barrier for the passage of materials, supplies, or equipment.

2. A hands-free sink must be provided near the doors of the cabinet room(s) and the inner change rooms. A sink must be provided in the outer change room. All sinks in the room(s) containing the Class III BSC must be connected to the wastewater decontamination system.
3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to liquids and chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

All penetrations in the internal shell of the laboratory and inner change room must be sealed.

Openings around doors into the cabinet room and inner change room must be minimized and capable of being sealed to facilitate decontamination.

All drains in ABSL-4 laboratory area floor must be connected directly to the liquid waste decontamination system.

Services and plumbing that penetrate the laboratory walls, floors or ceiling, must be installed to ensure that no backflow from the laboratory occurs. Services must be sealed and be provided with redundant backflow prevention. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and are sealed up to the second filter.

Decontamination of the entire cabinet must be performed using a validated gaseous or vapor method when there have been significant changes in cabinet usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment. Selection of the appropriate materials and methods used for decontamination must be based on the risk assessment of the biological agents in use.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture should be covered with a non-porous material that can be easily decontaminated.

5. Windows must be break-resistant and sealed.
6. If Class II BSCs are needed in the cabinet laboratory, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the cabinet room. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.
8. An eyewash station must be readily available in the laboratory.
9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3-Ag labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the ABSL-4 laboratory at negative pressure to surrounding areas and provide differential pressure/directional airflow between adjacent areas within the laboratory.

Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified.

Supply air to and exhaust air from the cabinet room and fumigation/decontamination chambers must pass through HEPA filter(s). The air exhaust discharge must be located away from occupied spaces and building air intakes.

All HEPA filters should be located as near as practicable to the cabinet laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings should be designed to allow for *in situ* decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers; decontamination ports, and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

Class III BSCs must be directly and independently exhausted through two HEPA filters in series. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet room(s). Access to the exit side of the pass through shall be limited to those individuals authorized to be in the ABSL-4 laboratory.
12. Liquid effluents from cabinet room sinks, floor drains, autoclave chambers, and other sources within the cabinet room must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often as required by institutional policy.

Effluents from showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door autoclave must be provided for decontaminating waste or other materials passing out of the cabinet room. Autoclaves that open outside of the laboratory must be sealed to the wall. This bioseal must be durable, airtight, and sealed to the wall. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door can only be opened after the autoclave decontamination cycle has been completed.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that over-pressurization cannot release unfiltered air or steam exposed to infectious material to the environment.

14. The ABSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.
15. Appropriate communication systems must be provided between the ABSL-4 laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and access/egress must be considered.

Suit Laboratory

1. The ABSL-4 suit laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

Entry to this laboratory must be through an airlock fitted with airtight doors. Personnel who enter this laboratory must wear a positive pressure suit ventilated by a life support system with HEPA filtered breathing air. The breathing air system must have redundant compressors, failure alarms and an emergency backup system.

Rooms in the facility must be arranged to ensure sequential passage through the chemical shower, inner (dirty) change room, personal shower, and outer (clean) changing area upon exit.

A chemical shower must be provided to decontaminate the surface of the positive pressure suit before the worker leaves the ABSL-4 laboratory. In the event of an emergency exit or failure of chemical shower, a method for decontaminating positive pressure suits, such as a gravity fed supply of chemical disinfectant, is needed.

An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit, and security systems should be on a UPS.

A double-door autoclave, dunk tank, or fumigation chamber must be provided at the containment barrier for the passage of materials, supplies, or equipment.

2. Sinks inside the ABSL-4 laboratory must be placed near procedure areas, contain traps, and be connected to the wastewater decontamination system.

3. Walls, floors, and ceilings of the ABSL-4 laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to liquids and chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

All penetrations in the internal shell of the laboratory, suit storage room and the inner change room must be sealed.

Drains, if present, in the laboratory floor must be connected directly to the liquid waste decontamination system. Sewer vents and other service lines must be protected by two HEPA filters in series and have protection against insect and animal intrusion.

Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

Decontamination of the entire laboratory must be performed using a validated gaseous or vapor method when there have been significant changes in laboratory usage, before major renovations or maintenance shut downs, and in other situations, as determined by risk assessment.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning, decontamination and unencumbered movement of personnel. Chairs and other furniture should be covered with a non-porous material that can be easily decontaminated. Sharp edges and corners should be avoided.
5. Windows must be break-resistant and sealed.
6. BSCs and other primary containment barrier systems must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the ABSL-4 laboratory. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.

8. An eyewash station must be readily available in the laboratory area for use during maintenance and repair activities.
9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3-Ag labs) may share ventilation systems if gas tight dampers and HEPA filters isolate each individual laboratory system.

The supply and exhaust components of the ventilation system must be designed to maintain the BSL-4/ABSL-4 laboratory at negative pressure to surrounding areas and provide correct differential pressure between adjacent areas within the laboratory.

Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified.

Supply air to the ABSL-4 laboratory, including the decontamination shower, must pass through a HEPA filter. All exhaust air from the BSL-4/ABSL-4 suit laboratory, decontamination shower and fumigation or decontamination chambers must pass through two HEPA filters, in series before discharge to the outside. The exhaust air discharge must be located away from occupied spaces and air intakes.

All HEPA filters must be located as near as practicable to the areas where infectious materials and/or animals are housed or are manipulated in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

The HEPA filter housings are designed to allow for *in situ* decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers; decontamination ports; and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. Biological safety cabinets can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly to the outside through an independent, direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the ABSL-4 laboratory. Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the ABSL-4 laboratory.
12. Liquid effluents from chemical showers, sinks, floor drains, autoclave chambers, and other sources within the laboratory must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often as required by institutional policy.

Effluents from personal body showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the wall through which the autoclave passes. This bioseal must be durable and airtight. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.

The size of the autoclave should be sufficient to accommodate the intended usage, equipment size, and potential future increases in cage size. Autoclaves should facilitate isolation for routine servicing.

Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that over-pressurization cannot release unfiltered air or steam exposed to infectious material to the environment.

14. The ABSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified. Verification criteria should be modified as necessary by operational experience.

Consider placing ABSL-4 areas away from exterior walls of buildings to minimize the impact from the outside environmental and temperatures.

15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and access/egress should be considered.

References

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4. National Research Council; Institute for Laboratory Animal Research. Occupational health and safety in the care and use of nonhuman primates. Washington, DC: National Academy Press; 2003.
5. National Institutes of Health, Office of Laboratory Animal Welfare. Public Health Service policy on humane care and use of laboratory animals, Bethesda (MD); The National Institutes of Health (US); 2000.

Table 3. Summary of Recommended Animal Biosafety Levels for Activities in which Experimentally or Naturally Infected Vertebrate Animals are Used

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species <ul style="list-style-type: none"> ■ PPE: Laboratory coats and gloves; eye, face protection, as needed 	Standard animal facility: <ul style="list-style-type: none"> ■ No recirculation of exhaust air ■ Directional air flow recommended ■ Hand washing sink is available
2	<ul style="list-style-type: none"> ■ Agents associated with human disease ■ Hazard: percutaneous injury, ingestion, mucous membrane exposure 	ABSL-1 practice plus: <ul style="list-style-type: none"> ■ Limited access ■ Biohazard warning signs ■ "Sharps" precautions ■ Biosafety manual ■ Decontamination of all infectious wastes and animal cages prior to washing 	ABSL-1 equipment plus primary barriers: <ul style="list-style-type: none"> ■ Containment equipment appropriate for animal special ■ PPE: Laboratory coats, gloves, face, eye and respiratory protection, as needed 	ABSL-1 plus: <ul style="list-style-type: none"> ■ Autoclave available ■ Hand washing sink available ■ Mechanical cage washer recommended ■ Negative airflow into animal and procedure rooms recommended
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	ABSL-2 practice plus: <ul style="list-style-type: none"> ■ Controlled access ■ Decontamination of clothing before laundering ■ Cages decontaminated before bedding is removed ■ Disinfectant foot bath as needed 	ABSL-2 equipment plus: <ul style="list-style-type: none"> ■ Containment equipment for housing animals and cage dumping activities ■ Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols ■ PPE: Appropriate respiratory protection 	ABSL-2 facility plus: <ul style="list-style-type: none"> ■ Physical separation from access corridors ■ Self-closing, double-door access ■ Sealed penetrations ■ Sealed windows ■ Autoclave available in facility ■ Entry through ante-room or airlock ■ Negative airflow into animal and procedure rooms ■ Hand washing sink near exit of animal or procedure room
4	<ul style="list-style-type: none"> ■ Dangerous/exotic agents which pose high risk of aerosol transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments ■ Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level ■ Related agents with unknown risk of transmission 	ABSL-3 practices plus: <ul style="list-style-type: none"> ■ Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting ■ All wastes are decontaminated before removal from the facility 	ABSL-3 equipment plus: <ul style="list-style-type: none"> ■ Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure suit) used for all procedures and activities 	ABSL-3 facility plus: <ul style="list-style-type: none"> ■ Separate building or isolated zone ■ Dedicated supply and exhaust, vacuum, and decontamination systems ■ Other requirements outlined in the text

Section VI—Principles of Laboratory Biosecurity

Since the publication of the 4th edition of BMBL in 1999, significant events have brought national and international scrutiny to the area of laboratory security. These events, including the anthrax attacks on U.S. citizens in October 2001 and the subsequent expansion of the United States Select Agent regulations in December 2003, have led scientists, laboratory managers, security specialists, biosafety professionals, and other scientific and institutional leaders to consider the need for developing, implementing and/or improving the security of biological agents and toxins within their facilities. Appendix F of BMBL 4th edition provided a brief outline of issues to consider in developing a security plan for biological agents and toxins capable of serious or fatal illness to humans or animals. In December 2002, Appendix F was updated and revised as a security and emergency response guidance for laboratories working with select agents.¹ Section VI replaces the previous appendices. The current Appendix F discusses Select Agent and Toxin regulations.

This section describes laboratory biosecurity planning for microbiological laboratories. As indicated below, laboratories with good biosafety programs already fulfill many of the basic requirements for security of biological materials. For laboratories not handling select agents, the access controls and training requirements specified for BSL-2 and BSL-3 in BMBL may provide sufficient security for the materials being studied. Security assessments and additional security measures should be considered when select agents, other agents of high public health and agriculture concern, or agents of high commercial value such as patented vaccine candidates, are introduced into the laboratory.

The recommendations presented in this section are advisory. Excluding the Select Agent regulations, there is no current federal requirement for the development of a biosecurity program. However, the application of these principles and the assessment process may enhance overall laboratory management. Laboratories that fall under the Select Agent regulations should consult Appendix F (42 CFR part 73; 7 CFR 331 and 9 CFR 121).^{4,5,6}

The term “biosecurity” has multiple definitions. In the animal industry, the term biosecurity relates to the protection of an animal colony from microbial contamination. In some countries, the term biosecurity is used in place of the term biosafety. For the purposes of this section the term “biosecurity” will refer to the protection of microbial agents from loss, theft, diversion or intentional misuse. This is consistent with current WHO and American Biological Safety Association (ABSA) usage of this term.^{2,3}

Security is not a new concept in biological research and medical laboratories. Several of the security measures discussed in this section are embedded in the biosafety levels that serve as the foundation for good laboratory practices throughout the biological laboratory community. Most biomedical and

microbiological laboratories do not have select agents or toxins, yet maintain control over and account for research materials, protect relevant sensitive information, and work in facilities with access controls commensurate with the potential public health and economic impact of the biological agents in their collections. These measures are in place in most laboratories that apply good laboratory management practices and have appropriate biosafety programs.

Biosafety and Biosecurity

Biosafety and biosecurity are related, but not identical, concepts. Biosafety programs reduce or eliminate exposure of individuals and the environment to potentially hazardous biological agents. Biosafety is achieved by implementing various degrees of laboratory control and containment, through laboratory design and access restrictions, personnel expertise and training, use of containment equipment, and safe methods of managing infectious materials in a laboratory setting.

The objective of biosecurity is to prevent loss, theft or misuse of microorganisms, biological materials, and research-related information. This is accomplished by limiting access to facilities, research materials and information. While the objectives are different, biosafety and biosecurity measures are usually complementary.

Biosafety and biosecurity programs share common components. Both are based upon risk assessment and management methodology; personnel expertise and responsibility; control and accountability for research materials including microorganisms and culture stocks; access control elements; material transfer documentation; training; emergency planning; and program management.

Biosafety and biosecurity program risk assessments are performed to determine the appropriate levels of controls within each program. Biosafety looks at appropriate laboratory procedures and practices necessary to prevent exposures and occupationally-acquired infections, while biosecurity addresses procedures and practices to ensure that biological materials and relevant sensitive information remain secure.

Both programs assess personnel qualifications. The biosafety program ensures that staff are qualified to perform their jobs safely through training and documentation of technical expertise. Staff must exhibit the appropriate level of professional responsibility for management of research materials by adherence to appropriate materials management procedures. Biosafety practices require laboratory access to be limited when work is in progress. Biosecurity practices ensure that access to the laboratory facility and biological materials are limited and controlled as necessary. An inventory or material management process for control and tracking of biological stocks or other sensitive materials is also a component of both programs. For biosafety, the shipment of infectious biological materials must adhere to safe packaging, containment and appropriate transport procedures, while biosecurity ensures that transfers are controlled, tracked and

documented commensurate with the potential risks. Both programs must engage laboratory personnel in the development of practices and procedures that fulfill the biosafety and biosecurity program objectives but that do not hinder research or clinical/diagnostic activities. The success of both of these programs hinges on a laboratory culture that understands and accepts the rationale for biosafety and biosecurity programs and the corresponding management oversight.

In some cases, biosecurity practices may conflict with biosafety practices, requiring personnel and management to devise policies that accommodate both sets of objectives. For example, signage may present a conflict between the two programs. Standard biosafety practice requires that signage be posted on laboratory doors to alert people to the hazards that may be present within the laboratory. The biohazard sign normally includes the name of the agent, specific hazards associated with the use or handling of the agent and contact information for the investigator. These practices may conflict with security objectives. Therefore, biosafety and biosecurity considerations must be balanced and proportional to the identified risks when developing institutional policies.

Designing a biosecurity program that does not jeopardize laboratory operations or interfere with the conduct of research requires a familiarity with microbiology and the materials that require protection. Protecting pathogens and other sensitive biological materials while preserving the free exchange of research materials and information may present significant institutional challenges. Therefore, a combination or tiered approach to protecting biological materials, commensurate with the identified risks, often provides the best resolution to conflicts that may arise. However, in the absence of legal requirements for a biosecurity program, the health and safety of laboratory personnel and the surrounding environment should take precedence over biosecurity concerns.

Risk Management Methodology

A risk management methodology can be used to identify the need for a biosecurity program. A risk management approach to laboratory biosecurity 1) establishes which, if any, agents require biosecurity measures to prevent loss, theft, diversion, or intentional misuse, and 2) ensures that the protective measures provided, and the costs associated with that protection, are proportional to the risk. The need for a biosecurity program should be based on the possible impact of the theft, loss, diversion, or intentional misuse of the materials, recognizing that different agents and toxins will pose different levels of risk. Resources are not infinite. Biosecurity policies and procedures should not seek to protect against every conceivable risk. The risks need to be identified, prioritized and resources allocated based on that prioritization. Not all institutions will rank the same agent at the same risk level. Risk management methodology takes into consideration available institutional resources and the risk tolerance of the institution.

Developing a Biosecurity Program

Management, researchers and laboratory supervisors must be committed to being responsible stewards of infectious agents and toxins. Development of a biosecurity program should be a collaborative process involving all stakeholders. The stakeholders include but are not limited to: senior management; scientific staff; human resource officials; information technology staff; and safety, security and engineering officials. The involvement of organizations and/or personnel responsible for a facility's overall security is critical because many potential biosecurity measures may already be in place as part of an existing safety or security program. This coordinated approach is critical in ensuring that the biosecurity program provides reasonable, timely and cost-effective solutions addressing the identified security risks without unduly affecting the scientific or business enterprise or provision of clinical and/or diagnostic services.

The need for a biosecurity program should reflect sound risk management practices based on a site-specific risk assessment. A biosecurity risk assessment should analyze the probability and consequences of loss, theft and potential misuse of pathogens and toxins.⁷ Most importantly, the biosecurity risk assessment should be used as the basis for making risk management decisions.

Example Guidance: A Biosecurity Risk Assessment and Management Process

Different models exist regarding biosecurity risk assessment. Most models share common components such as asset identification, threat, vulnerability and mitigation. What follows is one example of how a biosecurity risk assessment may be conducted. In this example, the entire risk assessment and risk management process may be divided into five main steps, each of which can be further subdivided: 1) identify and prioritize biologicals and/or toxins; 2) identify and prioritize the adversary/threat to biologicals and/or toxins; 3) analyze the risk of specific security scenarios; 4) design and develop an overall risk management program; and 5) regularly evaluate the institution's risk posture and protection objectives. Example guidance for these five steps is provided below.

Step 1: Identify and Prioritize Biological Materials

- Identify the biological materials that exist at the institution, form of the material, location and quantities, including non-replicating materials (i.e., toxins).
- Evaluate the potential for misuse of these biologic materials.
- Evaluate the consequences of misuse of these biologic materials.
- Prioritize the biologic materials based on the consequences of misuse (i.e., risk of malicious use).

At this point, an institution may find that none of its biologic materials merit the development and implementation of a separate biosecurity program or the existing security at the facility is adequate. In this event, no additional steps would need to be completed.

Step 2: Identify and Prioritize the Threat to Biological Materials

- Identify the types of “Insiders” who may pose a threat to the biologic materials at the institution.
- Identify the types of “Outsiders” (if any) who may pose a threat to the biologic materials at the institution.
- Evaluate the motive, means, and opportunity of these various potential adversaries.

Step 3: Analyze the Risk of Specific Security Scenarios

- Develop a list of possible biosecurity scenarios, or undesired events that could occur at the institution (each scenario is a combination of an agent, an adversary, and an action). Consider:
 - access to the agent within your laboratory;
 - how the undesired event could occur;
 - protective measures in place to prevent occurrence;
 - how the existing protection measures could be breached (i.e., vulnerabilities).
- Evaluate the probability of each scenario materializing (i.e., the likelihood) and its associated consequences. Assumptions include:
 - although a wide range of threats are possible, certain threats are more probable than others;
 - all agents/assets are not equally attractive to an adversary; valid and credible threats, existing precautions, and the potential need for select enhanced precautions are considered.
- Prioritize or rank the scenarios by risk for review by management.

Step 4: Develop an Overall Risk Management Program

- Management commits to oversight, implementation, training and maintenance of the biosecurity program.
- Management develops a biosecurity risk statement, documenting which biosecurity scenarios represent an unacceptable risk and must be mitigated versus those risks appropriately handled through existing protection controls.

- Management develops a biosecurity plan to describe how the institution will mitigate those unacceptable risks including:
 - a written security plan, standard operating procedures, and incident response plans;
 - written protocols for employee training on potential hazards, the biosecurity program and incident response plans.
- Management ensures necessary resources to achieve the protection measures documented in the biosecurity plan.

Step 5: Re-evaluate the Institution's Risk Posture and Protection Objectives

- Management regularly reevaluates and makes necessary modifications to the:
 - biosecurity risk statement;
 - biosecurity risk assessment process;
 - the institution's biosecurity program/plan;
 - the institution's biosecurity systems.
- Management assures the daily implementation, training and annual re-evaluation of the security program.

Elements of a Biosecurity Program

Many facilities may determine that existing safety and security programs provide adequate mitigation for the security concerns identified through biosecurity risk assessment. This section offers examples and suggestions for components of a biosecurity program should the risk assessment reveal that further protections may be warranted. Program components should be site-specific and based upon organizational threat/vulnerability assessment and as determined appropriate by facility management. Elements discussed below should be implemented, as needed, based upon the risk assessment process. They should not be construed as "minimum requirements" or "minimum standards" for a biosecurity program.

Program Management

If a biosecurity plan is implemented, institutional management must support the biosecurity program. Appropriate authority must be delegated for implementation and the necessary resources provided to assure program goals are being met. An organizational structure for the biosecurity program that clearly defines the chain of command, roles, and responsibilities should be distributed to the staff. Program management should ensure that biosecurity plans are created, exercised, and revised as needed. The biosecurity program should be integrated into relevant institutional policies and plans.

Physical Security—Access Control and Monitoring

The physical security elements of a laboratory biosecurity program are intended to prevent the removal of assets for non-official purposes. An evaluation of the physical security measures should include a thorough review of the building and premises, the laboratories, and biological material storage areas. Many requirements for a biosecurity plan may already exist in a facility's overall security plan.

Access should be limited to authorized and designated employees based on the need to enter sensitive areas. Methods for limiting access could be as simple as locking doors or having a card key system in place. Evaluations of the levels of access should consider all facets of the laboratory's operations and programs (e.g., laboratory entrance requirements, freezer access). The need for entry by visitors, laboratory workers, management officials, students, cleaning/maintenance staff, and emergency response personnel should be considered.

Personnel Management

Personnel management includes identifying the roles and responsibilities for employees who handle, use, store and transport dangerous pathogens and/or other important assets. The effectiveness of a biosecurity program against identified threats depends, first and foremost, on the integrity of those individuals who have access to pathogens, toxins, sensitive information and/or other assets. Employee screening policies and procedures are used to help evaluate these individuals. Policies should be developed for personnel and visitor identification, visitor management, access procedures, and reporting of security incidents.

Inventory and Accountability

Material accountability procedures should be established to track the inventory, storage, use, transfer and destruction of dangerous biological materials and assets when no longer needed. The objective is to know what agents exist at a facility, where they are located, and who is responsible for them. To achieve this, management should define: 1) the materials (or forms of materials) subject to accountability measures; 2) records to be maintained, update intervals and timelines for record maintenance; 3) operating procedures associated with inventory maintenance (e.g., how material is identified, where it can be used and stored); and 4) documentation and reporting requirements.

It is important to emphasize that microbiological agents are capable of replication and are often expanded to accommodate the nature of the work involving their use. Therefore, knowing the exact "working" quantity of organisms at any given time may be impractical. Depending on the risks associated with a pathogen or toxin, management can designate an individual who is accountable, knowledgeable about the materials in use, and responsible for security of the materials under his or her control.

Information Security

Policies should be established for handling sensitive information associated with the biosecurity program. For the purpose of these policies, “sensitive information” is that which is related to the security of pathogens and toxins, or other critical infrastructure information. Examples of sensitive information may include facility security plans, access control codes, agent inventories and storage locations. Discussion of information security in this section does not pertain to information which has been designated “classified” by the United States pursuant to Executive Order 12958, as amended, and is governed by United States law or to research-related information which is typically unregulated or unrestricted through the peer review and approval processes.

The objective of an information security program is to protect information from unauthorized release and ensure that the appropriate level of confidentiality is preserved. Facilities should develop policies that govern the identification, marking and handling of sensitive information. The information security program should be tailored to meet the needs of the business environment, support the mission of the organization, and mitigate the identified threats. It is critical that access to sensitive information be controlled. Policies for properly identifying and securing sensitive information including electronic files and removable electronic media (e.g., CDs, computer drives) should be developed.

Transport of Biological Agents

Material transport policies should include accountability measures for the movement of materials within an institution (e.g., between laboratories, during shipping and receiving activities) and outside of the facility (e.g., between institutions or locations). Transport policies should address the need for appropriate documentation and material accountability and control procedures for pathogens in transit between locations. Transport security measures should be instituted to ensure that appropriate authorizations have been received and that adequate communication between facilities has occurred before, during, and after transport of pathogens or other potentially hazardous biological materials. Personnel should be adequately trained and familiar with regulatory and institutional procedures for proper containment, packaging, labeling, documentation and transport of biological materials.

Accident, Injury and Incident Response Plans

Laboratory security policies should consider situations that may require emergency responders or public safety personnel to enter the facility in response to an accident, injury or other safety issue or security threat. The preservation of human life, the safety and health of laboratory employees and the surrounding community must take precedence in an emergency over biosecurity concerns. Facilities are encouraged to coordinate with medical, fire, police and other emergency officials when preparing emergency and security breach response

plans. Standard Operation Procedures (SOPs) should be developed that minimize the potential exposure of responding personnel to potentially hazardous biological materials. Laboratory emergency response plans should be integrated with relevant facility-wide or site-specific security plans. These plans should also consider such adverse events as bomb threats, natural disasters and severe weather, power outages, and other facility emergencies that may introduce security threats.

Reporting and Communication

Communication is an important aspect of a biosecurity program. A “chain-of-notification” should be established in advance of an actual event. This communication chain should include laboratory and program officials, institution management, and any relevant regulatory or public authorities. The roles and responsibilities of all involved officials and programs should be clearly defined. Policies should address the reporting and investigation of potential security breaches (e.g., missing biological agents, unusual or threatening phone calls, unauthorized personnel in restricted areas).

Training and Practice Drills

Biosecurity training is essential for the successful implementation of a biosecurity program. Program management should establish training programs that inform and educate individuals regarding their responsibilities within the laboratory and the institution. Practice drills should address a variety of scenarios such as loss or theft of materials, emergency response to accidents and injuries, incident reporting and identification of and response to security breaches. These scenarios may be incorporated into existing emergency response drills such as fire drills or building evacuation drills associated with bomb threats. Incorporating biosecurity measures into existing procedures and response plans often provides efficient use of resources, saves time and can minimize confusion during emergencies.

Security Updates and Re-evaluations

The biosecurity risk assessment and program should be reviewed and updated routinely and following any biosecurity-related incident. Reevaluation is a necessary and on-going process in the dynamic environments of today’s biomedical and research laboratories. Biosecurity program managers should develop and conduct biosecurity program audits and implement corrective actions as needed. Audit results and corrective actions should be documented. The appropriate program officials should maintain records.

Select Agents

If an entity possesses, uses or transfers select agents, it must comply with all requirements of the National Select Agent Program. See Appendix F for

additional guidance on the CDC and USDA Select Agent Programs (42 CFR Part 73; 7 CFR 331 and 9 CFR 121).

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Section VII—Occupational Health and Immunoprophylaxis

The goal of medical support services in a biomedical research setting is to promote a safe and healthy workplace. This is accomplished by limiting opportunities for exposure, promptly detecting and treating exposures, and using information gained from work injuries to further enhance safety precautions. Occupational health and safety in biomedical research settings is a responsibility shared by healthcare providers, safety specialists, principal investigators, employers, and workplace personnel. Optimal worker protection depends on effective, ongoing collaboration among these groups. Supervisors, working with personnel representatives, should describe workers' proposed tasks and responsibilities. First line supervisors and safety professionals should identify the potential worksite health hazards. Principal investigators may serve as subject matter experts. The health provider should design medical support services in consultation with representatives from the institutional environmental health and safety program and the principal investigators. Workers should be fully informed of the available medical support services and encouraged to utilize them. Requisite occupational medical services are described below and expanded discussions of the principles of effective medical support services are available in authoritative texts.^{1,2}

Services offered by the medical support team should be designed to be in compliance with United States Department of Labor (DOL), OSHA regulations, patient confidentiality laws, and the Americans with Disabilities Act of 1990.³⁻⁸ Medical support services should be based upon detailed risk assessments and tailored to meet the organization's needs. Risk assessments should define potential hazards and exposures by job responsibility. They should be provided for all personnel regardless of employment status. Contracted workers, students, and visitors should be provided occupational medical care by their employer or sponsor equivalent to that provided by the host institution for exposures, injuries, or other emergencies experienced at the worksite.

Occupational medical services may be provided through a variety of arrangements (e.g., in-house or community based) as long as the service is readily available and allows timely, appropriate evaluation and treatment. The interaction between worker, healthcare provider and employer may be complex, such as a contract worker who uses his own medical provider or uses contract medical services. Thus, plans for providing medical support for workers should be completed before work actually begins. The medical provider must be knowledgeable about the nature of potential health risks in the work environment and have access to expert consultation.

Prevention is the most effective approach to managing biohazards. Prospective workers should be educated about the biohazards to which they may be occupationally exposed, the types of exposures that place their health at risk, the nature and significance of such risks, as well as the appropriate first aid and follow up for potential exposures. That information should be reinforced

annually, at the time of any significant change in job responsibility, and following recognized and suspected exposures.⁹⁻¹¹

Medical support services for biomedical research facilities should be evaluated annually. Joint annual review of occupational injury and illness reports by healthcare providers and environmental health and safety representatives can assist revision of exposure prevention strategies to minimize occupational health hazards that cannot be eliminated.

Occupational Health Support Service Elements

Preplacement Medical Evaluations

Workers who may be exposed to human pathogens should receive a preplacement medical evaluation. Healthcare providers should be cognizant of potential hazards encountered by the worker. A description of the requirements for the position and an understanding of the potential health hazards present in the work environment, provided by the worker's supervisor, should guide the evaluation. The healthcare provider should review the worker's previous and ongoing medical problems, current medications, allergies to medicines, animals, and other environmental proteins, and prior immunizations. With that information, the healthcare provider determines what medical services are indicated to permit the individual to safely assume the duties of the position. Occasionally, it may be useful to review pre-existing medical records to address specific concerns regarding an individual's medical fitness to perform the duties of a specific position. If pre-existing medical records are unavailable or are inadequate, the healthcare provider may need to perform a targeted medical exam. Comprehensive physical examinations are rarely indicated. During the visit, the healthcare provider should inform the worker of potential health hazards in the work area and review steps that should be taken in the event of an accidental exposure. This visit also establishes a link with the medical support services provider.

When occupational exposure to human pathogens is a risk, employers should consider collecting and storing a serum specimen prior to the initiation of work with the agent. It can be used to establish baseline sero-reactivity, should additional blood samples be collected for serological testing subsequent to a recognized or suspected exposure.

Occasionally, it is desirable to determine an individual's vulnerability to infection with specific agents prior to assigning work responsibilities. Some occupational exposures present substantially more hazard to identifiable sub-populations of workers. Immunodeficient workers or non-immune pregnant female workers may experience devastating consequences from exposures that pose a chance of risk to pregnant women with prior immunity and other immunocompetent workers (e.g., cytomegalovirus or toxoplasmosis). Serologic testing should be used to document baseline vulnerability to specific infections to which the worker might

be exposed, and non-immune workers should be adequately informed about risks. In specific settings, serologic documentation that individual workers have pre-existing immunity to specific infections also may be required for the protection of research animals.¹⁰

Vaccines

Commercial vaccines should be made available to workers to provide protection against infectious agents to which they may be occupationally exposed.¹²⁻¹⁶ The Advisory Committee on Immunization Practices (ACIP) provides expert advice to the Secretary of the DHHS, the Assistant Secretary for Health, and the CDC on the most effective means to prevent vaccine-preventable diseases and to increase the safe usage of vaccines and related biological products. The ACIP develops recommendations for the routine administration of vaccines to pediatric and adult populations, and schedules regarding the appropriate periodicity, dosage, and contraindications. The ACIP is the only entity in the federal government that makes such recommendations. The ACIP is available at the CDC Web site: www.cdc.gov.

If the potential consequences of infection are substantial and the protective benefit from immunization is proven, acceptance of such immunization may be a condition for employment. Current, applicable vaccine information statements must be provided whenever a vaccine is administered. Each worker's immunization history should be evaluated for completeness and currency at the time of employment and re-evaluated when the individual is assigned job responsibilities with a new biohazard.

When occupational exposure to highly pathogenic agents is possible and no commercial vaccine is available, it may be appropriate to immunize workers using vaccines or immune serum preparations that are investigational, or for which the specific indication constitutes an off-label use. Use of investigational products, or of licensed products for off-label indications must be accompanied by adequate informed consent outlining the limited availability of information on safety and efficacy. Use of investigational products should occur through Investigational New Drug (IND) protocols providing safety oversight by both the Food and Drug Administration (FDA) and appropriate Institutional Human Subjects Research Protection Committees.^{17,18} Recommendation of investigational products, as well as commercial vaccines that are less efficacious, associated with high rates of local or systemic reactions, or that produce increasingly severe reactions with repeated use, should be considered carefully. Receipt of such vaccines is rarely justified as a job requirement.

Investigational vaccines for eastern equine encephalomyelitis (EEE) virus, Venezuelan equine encephalitis (VEE) virus, western equine encephalomyelitis (WEE) virus, and Rift Valley fever viruses (RVFV), may be available in limited

quantities and administered on-site at the Special Immunization Program, United States Army Medical Research Institute of Infectious Diseases (USAMRIID).

Periodic Medical Evaluations

Routine, periodic medical evaluations generally are not recommended; however, limited periodic medical evaluations or medical clearances targeted to job requirements may occasionally be warranted (e.g., respirator usage).³ In special circumstances, it may be appropriate to offer periodic laboratory testing to workers with substantial risk of exposure to infectious agents to detect pre-clinical or sub-clinical evidence for an occupationally acquired infection. Before asymptomatic workers without specific exposures are tested for seroreactivity, the benefit of such testing should be justified, plans for further investigation of indeterminate test results should be delineated, and clearly defined criteria for interpretation of results should be developed.

Medical Support for Occupational Illnesses and Injuries

Workers should be encouraged to seek medical evaluation for symptoms that they suspect may be related to infectious agents in their work area, without fear of reprisal. A high index of suspicion for potential occupational exposures should be maintained during any unexplained illness among workers or visitors to worksites containing biohazards. Modes of transmission, as well as the clinical presentation of infections acquired through occupational exposures, may differ markedly from naturally acquired infections. Fatal occupational infections have resulted from apparently trivial exposures. The healthcare provider should have a working understanding of the biohazards present in the workplace and remain alert for subtle evidence of infection and atypical presentations. A close working relationship with the research or clinical program in which the affected employee works is absolutely essential. In the event of injury, consultation between healthcare provider, employee, and the employee's supervisor is required for proper medical management and recordkeeping.

All occupational injuries, including exposures to human pathogens, should be reported to the medical support services provider. Strategies for responding to biohazard exposures should be formulated in advance. Proper post-exposure response is facilitated by exposure-specific protocols that define appropriate first aid, potential post-exposure prophylaxis options, recommended diagnostic tests, and sources of expert medical evaluation. These protocols should address how exposures that occur outside of regular work hours are handled and these protocols should be distributed to potential healthcare providers (e.g., local hospital emergency departments). In exceptional cases, the protocols should be reviewed with state and community public health departments. Emergency medical support training should be provided on a regular basis for both employees and healthcare providers.

The adequacy and timeliness of wound cleansing or other response after an exposure occurs may be the most critical determinant in preventing infection. First aid should be defined, widely promulgated, and immediately available to an injured worker. Barriers to subsequent medical evaluation and treatment should be identified and minimized to facilitate prompt, appropriate care. Laboratory SOPs should include a printed summary of the recommended medical response to specific exposures that can guide immediate response in the work place and that the injured worker can provide to the treating facility. The medical provider's description of the injury should include:

- The potential infectious agent.
- The mechanism and route of exposure (percutaneous, splash to mucous membranes or skin, aerosol, etc.).
- Time and place of the incident.
- Personal protective equipment used at the time of the injury.
- Prior first aid provided (e.g., nature and duration of cleaning and other aid, time that lapsed from exposure to treatment).
- Aspects of the worker's personal medical history relevant to risk of infection or complications of treatment.

First aid should be repeated if the initial adequacy is in question. Healthcare providers must use appropriate barrier precautions to avoid exposure to infectious agents and toxins.

In some instances, it may be possible to prevent or ameliorate illness through post-exposure prophylaxis. Protocols should be developed in advance that clearly identify the situations in which post-exposure prophylaxis are to be considered, the appropriate treatment, and the source of products and expert consultation. Accurate quantification of risk associated with all exposures is not possible, and the decision to administer post-exposure prophylaxis may have to be made quickly and in the absence of confirmatory laboratory testing. Post-exposure regimens may involve off-label use of licensed products (e.g., use of smallpox vaccine for workers exposed to monkeypox) in settings where there is insufficient experience to provide exact guidance on the safety or likely protective efficacy of the prophylactic regimen. Thus, protocols should exist that delineate the circumstances under which it would be appropriate to consider use of each product following exposure, as well as the limits of our understanding of the value of some post-exposure interventions. In these cases, consultations with subject matter experts are especially useful.

Estimating the significance of an exposure may be difficult, despite having established protocols. The clinician may need to make a "best-estimate" based

upon knowledge of similar agents, exposure circumstances, and advice received from knowledgeable experts. Appropriate post-exposure prophylactic response is always pathogen and exposure dependent, and may be host-factor dependent and influenced by immediate post-exposure management. Before prophylactic treatment is undertaken, confirm the likelihood that an exposure occurred, that prophylaxis is indicated and is not contraindicated by past medical history. Conveying this information to the injured worker requires clear, honest communication. The clinical risk assessment and treatment decision process should be carefully explained, the worker's questions addressed with relevant, preprinted educational materials provided. Prompt treatment should be provided, with a mutually agreed plan to follow the individual's clinical course.

The applicable workers compensation claim form should be provided with appropriate explanations for its completion. The supervisor must receive a description of the accident or incident, confirm the circumstances of the injury or exposure and provide relevant advice. The report also should be distributed to all other relevant parties, such as the safety professional. Each incident should receive prompt reconsideration of the initial risk assessment and reevaluation of current strategies to reduce the possibility of future exposures.

Post-exposure serologic testing may be useful, but it is important to determine how information obtained from serologic testing will be interpreted. It is also essential to collect serum specimens at the appropriate interval for a given situation. Assessment of sero-reactivity in exposed workers is most helpful when the results of specimens collected over time can be compared. Ideally specimens collected prior to, at the time of and several weeks following exposure, should be tested simultaneously and results compared to assess changes in the pattern of sero-reactivity. Serum collected too early after exposure may fail to react even when infection has occurred, because antibodies have not yet been produced in detectable quantities. When immediate institution of post-exposure prophylaxis may delay seroconversion, or when the agent to which the worker was exposed results in seroconversion completed over months (e.g., retroviruses), testing of specimens collected late after exposure is particularly important.

Testing of a single serum specimen is generally discouraged and can result in misinterpretation of nonspecific sero-reactivity. Evidence of sero-conversion or a significant (≥ 4 fold) increase in titer associated with a compatible clinical syndrome is highly suggestive of acute infection.

However, the significance of and appropriate response to sero-conversion in the absence of illness is not always clear. If sero-reactivity is evident in the earliest specimen, it is important to re-test that specimen in tandem with serum specimens archived prior to occupational exposure and/or collected serially over time to investigate whether a change in titer suggestive of new infection can be identified.

In some exposure situations, it may be appropriate to store serially collected serum samples, and to send them for testing as evidence of seroconversion only if symptoms develop that suggest an infection may have occurred (e.g., Monkey B virus exposures). Serum collected at the time of employment, and any other specimens not immediately tested should be stored frozen at a temperature of -20° C or lower in a freezer that does not experience freeze-thaw cycles. An inventory system should be established to ensure the accurate and timely retrieval of samples, while protecting patient privacy.

When investigational or other non-commercial assays are utilized, the importance of appropriate controls and the ability to compare serially collected specimens for quantification/characterization of reactivity is increased. The availability of aliquoted samples that allow additional testing may be essential to assist interpretation of ambiguous results. Caution should be taken to avoid placing more confidence in testing outcomes than can be justified by the nature of the assays.

Occupational Health in the BSL-4 Setting

Work with BSL-4 agents involves special challenges for occupational health. Infections of laboratory staff by such agents may be expected to result in serious or lethal disease for which limited treatment options exist. In addition, BSL-4 agents are frequently geographically exotic to the areas in which high containment labs are located but produce immediate public health concern if infections occur in laboratory staff. Potential (if unlikely) transmission from infected staff into the human or animal populations in the areas surrounding the laboratories may raise such concerns to higher levels. Thus, SOPs for BSL-4 settings require special attention to management of unexplained worker absence, including protocols for monitoring, medical evaluation, work-up, and follow-up of workers with unexplained nonspecific illness. Advance planning for the provision of medical care to workers potentially infected with BSL-4 agents is a fundamental component of an occupational health program for a BSL-4 facility.

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Section VIII—Agent Summary Statements

Section VIII-A: Bacterial Agents

Bacillus anthracis

Bacillus anthracis, a gram-positive, non-hemolytic, and non-motile bacillus, is the etiologic agent of anthrax, an acute bacterial disease of mammals, including humans. Like all members of the genus *Bacillus*, under adverse conditions *B. anthracis* has the ability to produce spores that allow the organism to persist for long periods until the return of more favorable conditions. Reports of suspected anthrax outbreaks date back to as early as 1250 BC. The study of anthrax and *B. anthracis* in the 1800s contributed greatly to our general understanding of infectious diseases. Much of Koch's postulates were derived from work on identifying the etiologic agent of anthrax. Louis Pasteur developed the first attenuated live vaccine for anthrax.

Most mammals are susceptible to anthrax; it mostly affects herbivores that ingest spores from contaminated soil and, to a lesser extent, carnivores that scavenge on the carcasses of diseased animals. Anthrax still occurs frequently in parts of central Asia and Africa. In the United States, it occurs sporadically in animals in parts of the West, Midwest and Southwest.

The infectious dose varies greatly from species to species and is route-dependent. The inhalation anthrax infectious dose (ID) for humans primarily has been extrapolated from inhalation challenges of nonhuman primates (NHP) or studies done in contaminated mills. Estimates vary greatly but the medium lethal dose (LD₅₀) is likely within the range of 2,500-55,000 spores.¹ It is believed that very few spores (10 or less) are required for cutaneous anthrax.²

Occupational Infections

Occupational infections are possible when in contact with contaminated animals, animal products or pure cultures of *B. anthracis*, and may include ranchers, veterinarians and laboratory workers. Numerous cases of laboratory-associated anthrax (primarily cutaneous) have been reported.^{3,4} Recent cases include suspected cutaneous anthrax in a laboratory worker in Texas and a cutaneous case in a North Dakota male who disposed of five cows that died of anthrax.^{5,6}

Natural Modes of Infection

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

significant public health problem in the United States, anthrax has become a bioterrorism concern. In 2001, 22 people were diagnosed with anthrax acquired from spores sent through the mail, including 11 cases of inhalation anthrax with five deaths and 11 cutaneous cases.⁷

Laboratory Safety and Containment Recommendations

B. anthracis may be present in blood, skin lesion exudates, cerebrospinal fluid, pleural fluid, sputum, and rarely, in urine and feces. The primary hazards to laboratory personnel are: direct and indirect contact of broken skin with cultures and contaminated laboratory surfaces, accidental parenteral inoculation and, rarely, exposure to infectious aerosols. Efforts should be made to avoid production of aerosols by working with infectious organisms in a BSC. In addition, all centrifugation should be done using aerosol-tight rotors that are opened within the BSC after each run.

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

Special Issues

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

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

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

Bordetella pertussis

Bordetella pertussis, an exclusively human respiratory pathogen of worldwide distribution, is the etiologic agent of whooping cough or pertussis. The organism is a fastidious, small gram-negative coccobacillus that requires highly specialized culture and transport media for cultivation in the laboratory. Its natural habitat is the human respiratory tract.

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

The agent may be present in high levels in respiratory secretions, and may be found in other clinical material, such as blood and lung tissue in its infrequent manifestation of septicemia and pneumonia, respectively.^{22,23} Because the natural mode of transmission is via the respiratory route, aerosol generation during the manipulation of cultures and contaminated clinical specimens generates the greatest potential hazard.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious clinical material and cultures. ABSL-2 practices and containment equipment should be employed for housing experimentally infected animals. Primary containment devices and equipment, including biological safety cabinets, safety centrifuge cups or safety centrifuges should be used for activities likely to

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

Special Issues

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

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

Brucella species

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

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

Brucella infects the blood and a wide variety of body tissues, including cerebral spinal fluid, semen, pulmonary excretions, placenta, and occasionally urine. Most laboratory-associated cases occur in research facilities and involve exposures to *Brucella* organisms grown in large quantities or exposure to placental tissues containing *Brucella*. Cases have occurred in clinical laboratory settings from sniffing bacteriological cultures²⁹ or working on open bench tops.³⁰ Aerosols from, or direct skin contact with, cultures or with infectious clinical specimens from animals (e.g., blood, body fluids, tissues) are commonly implicated in human infections. Aerosols generated during laboratory procedures have caused multiple cases per exposure.^{30,31} Mouth pipetting, accidental parenteral inoculations, and sprays into eyes, nose and mouth result in infection. The infectious dose of *Brucella* is 10-100 organisms by aerosol route and subcutaneous route in laboratory animals.^{32,33}

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

Special Issues

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

Select Agent *Brucella abortus*, *Brucella melitensis*, and *Brucella suis* are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

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

Burkholderia mallei

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

Occupational Infections

Glanders occurs almost exclusively among individuals who work with equine species and/or handle *B. mallei* cultures in the laboratory. *B. mallei* can be very infectious in the laboratory setting. The only reported case of human glanders in the United States over the past 50 years resulted from a laboratory exposure.³⁵ Modes of transmission may include inhalation and/or mucocutaneous exposure.

Natural Mode of Infection

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

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

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

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

Burkholderia pseudomallei

Burkholderia pseudomallei (formerly *Pseudomonas pseudomallei*) is a motile gram-negative, oxidase-positive rod that is found in soil and water environments of equatorial regions, including Southeast Asia, Northern Australia, Central America and South America. This organism is the causative agent of melioidosis, an unusual bacterial disease characterized by abscesses in tissues and organs. Victims of the disease frequently exhibit recrudescence months or years after the initial infection.

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

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

Special Issues

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

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

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

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

Occupational Infections

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

Natural Modes of Infection

Numerous domestic and wild animals, including poultry, pets, farm animals, laboratory animals, and wild birds are known reservoirs and are a potential source of infection for laboratory and animal care personnel. While the infective dose is not firmly established, ingestion of as few as 500-800 organisms has caused symptomatic infection.⁴⁹⁻⁵¹ Natural transmission usually occurs from ingestion of organisms in contaminated food or water and from direct contact with infected pets, farm animals, or infants.⁵²

Laboratory Safety and Containment Recommendations

Pathogenic *Campylobacter* sp. may occur in fecal specimens in large numbers. *C. fetus* subsp. *fetus* may also be present in blood, exudates from abscesses, tissues, and sputa. The primary laboratory hazards are ingestion and parenteral inoculation of *C. jejuni*. The significance of aerosol exposure is not known.

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

Special Issues

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

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

Chlamydia psittaci, *C. pneumoniae* (sometimes called *Chlamydophila psittaci* and *Chlamydophila pneumoniae*) and *C. trachomatis* are the three species of *Chlamydia* known to infect humans. Chlamydiae are nonmotile, gram-negative bacterial pathogens with obligate intracellular life cycles. These three species of *Chlamydia* vary in host spectrum, pathogenicity, and in the clinical spectrum of disease. *C. psittaci* is a zoonotic agent that commonly infects psittacine birds and is highly pathogenic for humans. *C. trachomatis* is historically considered an exclusively human pathogen and is the most commonly reported bacterial infection in the United States. *C. pneumoniae* is considered the least pathogenic species, often resulting in subclinical or asymptomatic infections in both animals and humans.

Occupational Infections

Chlamydial infections caused by *C. psittaci* and *C. trachomatis* lymphogranuloma venereum (LGV) strains were at one time among the most commonly reported laboratory-associated bacterial infections.²⁶ In cases reported before 1955⁴, the majority of infections were psittacosis, and these had the highest case fatality rate of laboratory-acquired infectious agents. The major sources of laboratory-associated psittacosis are contact with and exposure to infectious aerosols in the handling, care, or necropsy of naturally or experimentally infected birds. Infected mice and eggs also are important sources of *C. psittaci*. Most reports of laboratory-acquired infections with *C. trachomatis* attribute the infection to inhalation of large quantities of aerosolized organisms during purification or sonification procedures. Early reports commonly attributed infections to exposure to aerosols formed during nasal inoculation of mice or inoculation of egg yolk sacs and harvest of chlamydial elementary bodies. Infections are associated with fever, chills, malaise, and headache; a dry cough is also associated with *C. psittaci* infection. Some workers exposed to *C. trachomatis*

have developed conditions including mediastinal and supraclavicular lymphadenitis, pneumonitis, conjunctivitis, and keratitis.⁵³ Seroconversion to chlamydial antigens is common and often striking although early antibiotic treatment may prevent an antibody response.

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

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

C. psittaci may be present in the tissues, feces, nasal secretions and blood of infected birds, and in blood, sputum, and tissues of infected humans. *C. trachomatis* may be present in genital, bubo, and conjunctival fluids of infected humans. Exposure to infectious aerosols and droplets, created during the handling of infected birds and tissues, are the primary hazards to laboratory personnel working with *C. psittaci*. The primary laboratory hazards of *C. trachomatis* and *C. pneumoniae* are accidental parenteral inoculation and direct and indirect exposure of mucous membranes of the eyes, nose, and mouth to genital, bubo, or conjunctival fluids, cell culture materials, and fluids from infected cell cultures or eggs. Infectious aerosols, including those that may be created as a result of centrifuge malfunctions, also pose a risk for infection.

BSL-2 practices, containment equipment, and facilities are recommended for personnel working with clinical specimens and cultures or other materials known or suspected to contain the ocular or genital serovars (A through K) of *C. trachomatis* or *C. pneumoniae*.

BSL-3 practices, containment equipment, and facilities are recommended for activities involving the necropsy of infected birds and the diagnostic examination of tissues or cultures known to contain or be potentially infected with *C. psittaci* strains of avian origin. Wetting the feathers of infected birds with a detergent-disinfectant prior to necropsy can appreciably reduce the risk of aerosols of infected feces and nasal secretions on the feathers and external surfaces of the bird. Activities involving non-avian strains of *C. psittaci* may be performed in a BSL-2 facility as long as BSL-3 practices are followed, including but not limited to the use of primary containment equipment such as BSCs. ABSL-3 practices, containment equipment, and facilities and respiratory protection are recommended for personnel working with naturally or experimentally infected caged birds.

BSL-3 practices and containment equipment are recommended for activities involving work with culture specimens or clinical isolates known to contain or be potentially infected with the LGV serovars (L₁ through L₃) of *C. trachomatis*. Laboratory work with the LGV serovars of *C. trachomatis* can be conducted in a BSL-2 facility as long as BSL-3 practices are followed when handling potentially infectious materials, including but not limited to use of primary containment equipment such as BSCs.

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

ABSL-2 practices, containment equipment, and facilities are recommended for activities with animals that have been experimentally infected with genital serovars of *C. trachomatis* or *C. pneumoniae*.

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

Special Issues

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

Neurotoxin-producing *Clostridia* species

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

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

Neurotoxin producing *Clostridia* species or its toxin may be present in a variety of food products, clinical materials (serum, feces) and environmental samples (soil, surface water).⁵⁹ In addition, bacterial cultures may produce very high levels of toxin.⁶⁰ In healthy adults, it is typically the toxin and not the organism that causes disease. Risk of laboratory exposure is due to the presence of the toxin and not due to a potential of infection from the organisms that produce the toxin. Although spore-forming, there is no known risk to spore exposure except for the potential for the presence of residual toxin associated with pure spore preparations. Laboratory safety protocols should be developed with the focus on prevention of accidental exposure to the toxin produced by these *Clostridia* species.

BSL-2 practices, containment equipment, and facilities are recommended for activities that involve the organism or the toxin⁶¹ including the handling of potentially contaminated food. Solutions of sodium hypochlorite (0.1%) or sodium hydroxide (0.1N) readily inactivate the toxin and are recommended for decontamination of work surfaces and for spills. Autoclaving of contaminated materials also is appropriate.

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

Special Issues

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

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

Select Agent Neurotoxin producing *Clostridia* species are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

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

***Clostridium tetani* and Tetanus toxin**

Clostridium tetani is an anaerobic endospore-forming gram-positive rod found in the soil and an intestinal tract commensal. It produces a potent neurotoxin, tetanospasmin, which causes tetanus, an acute neurologic condition characterized by painful muscular contractions. Tetanospasmin is an exceedingly potent, high molecular weight protein toxin, consisting of a heavy chain (100kD) subunit that binds the toxin to receptors on neuronal cells and a light chain (50kD) subunit that blocks the release of inhibitory neural transmitter molecules within the central nervous system. The incidence of tetanus in the United States has declined steadily since the introduction of tetanus toxoid vaccines in the 1940's.⁶²

Occupational Infections

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

Natural Modes of Infection

Contamination of wounds by soil is the usual mechanism of transmission for tetanus. Of the 130 cases of tetanus reported to CDC from 1998 through 2000, acute injury (puncture, laceration, abrasion) was the most frequent predisposing condition. Elevated incidence rates also were observed for persons aged over 60 years, diabetics, and intravenous drug users.⁶³ When introduced into a suitable anaerobic or microaerophilic environment, *C. tetani* spores germinate and produce tetanospasmin. The incubation period ranges from 3 to 21 days. The observed symptoms are primarily associated with the presence of the toxin. Wound cultures are not generally useful for diagnosing tetanus.⁶⁴

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

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

Corynebacterium diphtheriae

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

Occupational Infections

Laboratory-associated infections with *C. diphtheriae* have been documented, but laboratory animal-associated infections have not been reported.^{4,66} Inhalation, accidental parenteral inoculation, and ingestion are the primary laboratory hazards.

Natural Modes of Infection

The agent may be present in exudates or secretions of the nose, throat (tonsil), pharynx, larynx, wounds, in blood, and on the skin. Travel to endemic areas or close contact with persons who have returned recently from such areas, increases risk.⁶⁷ Transmission usually occurs via direct contact with patients or carriers, and more rarely, with articles contaminated with secretions from infected people. Naturally occurring diphtheria is characterized by the development of grayish-white membranous lesions involving the tonsils, pharynx, larynx, or nasal mucosa. Systemic sequelae are associated with the production of diphtheria toxin. An effective vaccine has been developed for diphtheria and this disease has become a rarity in countries with vaccination programs.

Laboratory Safety and Containment Recommendations

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

Special Issues

Vaccines A licensed vaccine is available. The reader is advised to consult the current recommendations of the CIP.⁶⁵ While the risk of laboratory-associated diphtheria is low, the administration of an adult diphtheria-tetanus toxoid at 10-year intervals may further reduce the risk of illness to laboratory and animal care personnel.⁶²

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

Francisella tularensis

Francisella tularensis is a small gram-negative coccobacillus that is carried in numerous animal species, especially rabbits, and is the causal agent of tularemia (Rabbit fever, Deer fly fever, Ohara disease, or Francis disease) in humans. *F. tularensis* can be divided into three subspecies, *F. tularensis* (Type A), *F. holarctica* (Type B) and *F. novicida*, based on virulence testing, 16S sequence, biochemical reactions and epidemiologic features. Type A and Type B strains are highly infectious, requiring only 10-50 organisms to cause disease. Subspecies *F. novicida* is infrequently identified as the cause of human disease. Person-to-person transmission of tularemia has not been documented. The incubation period varies with the virulence of the strain, dose and route of introduction but ranges from 1-4 days with most cases exhibiting symptoms in 3-5 days.⁶⁸

Occupational Infections

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

Natural Modes of Infection

Tick bites, handling or ingesting infectious animal tissues or fluids, ingestion of contaminated water or food and inhalation of infective aerosols are the primary transmission modes in nature. Occasionally, infections have occurred from bites or scratches by carnivores with contaminated mouthparts or claws.

Laboratory Safety and Containment Recommendations

The agent may be present in lesion exudates, respiratory secretions, cerebrospinal fluid (CSF), blood, urine, tissues from infected animals, fluids from infected animals, and fluids from infected arthropods. Direct contact of skin or mucous membranes with infectious materials, accidental parenteral inoculation, ingestion, and exposure to aerosols and infectious droplets has resulted in infection. Infection has been more commonly associated with cultures than with clinical materials and infected animals.⁶⁹

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

Special Issues

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

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

Helicobacter species

Helicobacters are spiral or curved gram-negative rods isolated from gastrointestinal and hepatobiliary tracts of mammals and birds. There are currently 20 recognized species, including at least nine isolated from humans. Since its discovery in 1982, *Helicobacter pylori* has received increasing attention as an agent of gastritis.⁷⁰ The main habitat of *H. pylori* is the human gastric mucosa. Other *Helicobacter* spp. (*H. cinaedi*, *H. canadensis*, *H. canis*, *H. pullorum*, and *H. fennelliae*) may cause asymptomatic infection as well as proctitis, proctocolitis, enteritis and extraintestinal infections in humans.^{71,72} *H. cinaedi* has been isolated from dogs, cats and Syrian hamsters.

Occupational Infections

Both experimental and accidental LAI with *H. pylori* have been reported.^{73,74} Ingestion is the primary known laboratory hazard. The importance of aerosol exposures is unknown.

Natural Modes of Infection

Chronic gastritis and duodenal ulcers are associated with *H. pylori* infection. Epidemiologic associations have also been made with gastric adenocarcinoma. Human infection with *H. pylori* may be long in duration with few or no symptoms, or may present as an acute gastric illness. Transmission, while incompletely understood, is thought to be by the fecal-oral or oral-oral route.

Laboratory Safety and Containment Recommendations

H. pylori may be present in gastric and oral secretions and stool.⁷⁵ The enterohepatic helicobacters (e.g., *H. canadensis*, *H. canis*, *H. cinaedi*, *H. fennelliae*, *H. pullorum*, and *H. winthamensis*) may be isolated from stool specimens, rectal swabs, and blood cultures.⁷² Protocols involving homogenization or vortexing of gastric specimens have been described for the isolation of *H. pylori*.⁷⁶ Containment of potential aerosols or droplets should be incorporated in these procedures.

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

Special Issues

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

Legionella pneumophila and other Legionella-like Agents

Legionella are small, faintly staining gram-negative bacteria. They are obligately aerobic, slow-growing, nonfermentative organisms that have a unique requirement for L-cysteine and iron salts for *in vitro* growth. Legionellae are readily found in natural aquatic bodies and some species (*L. longbeachae*) have been recovered from soil.^{77,78} They are able to colonize hot-water tanks at a temperature range from 40 to 50°C. There are currently 48 known *Legionella* species, 20 of which have been associated with human disease. *L. pneumophila* is the species most frequently encountered in human infections.⁷⁹⁻⁸¹

Occupational Infections

Although laboratory-associated cases of legionellosis have not been reported in the literature, at least one case, due to presumed aerosol or droplet exposure during animal challenge studies with *L. pneumophila*, has been recorded.⁸² Experimental infections have been produced in guinea pigs, mice, rats, embryonated chicken eggs, and human or animal cell lines.⁸³ A fatal case of pneumonia due to *L. pneumophila* was diagnosed in a calf, but only 1.7% (2/112) of the other cattle in the herd had serological evidence of exposure to *Legionella*.⁸⁴ The disease was linked to exposure to a hot water system colonized with *Legionella*. Animal-to-animal transmission has not been demonstrated.

Natural Modes of Infection

Legionella is commonly found in environmental sources, typically in man-made warm water systems. The mode of transmission from these reservoirs is aerosolization, aspiration or direct inoculation into the airway.⁸⁵ Direct person-to-person transmission does not occur. The spectrum of illness caused by *Legionella* species ranges from a mild, self-limited flu-like illness (Pontiac fever) to a disseminated and often fatal disease characterized by pneumonia and respiratory failure (Legionnaires disease). Although rare, *Legionella* has been implicated in cases of sinusitis, cellulitis, pericarditis, and endocarditis.⁸⁶ Legionellosis may be either community-acquired or nosocomial. Risk factors include smoking, chronic lung disease, and immunosuppression. Surgery, especially involving transplantation, has been implicated as a risk factor for nosocomial transmission.

Laboratory Safety and Containment Recommendations

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

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of potentially infectious materials, including minimizing the potential for dissemination of the organism from cultures of organisms known to cause disease. ABSL-2 practices, containment equipment and facilities are recommended for activities with experimentally-infected animals. Routine processing of environmental water samples for *Legionella* may be performed with standard BSL-2 practices. For activities likely to produce extensive aerosols and when large quantities of the pathogenic organisms are manipulated, BSL-2 with BSL-3 practices is recommended.

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

Leptospira

The genus *Leptospira* is composed of spiral-shaped bacteria with hooked ends. Leptospirae are ubiquitous in nature, either free-living in fresh water or associated with renal infection in animals. Historically, these organisms have been classified into pathogenic (*L. interrogans*) and saprophytic (*L. biflexa*) groups, but recent studies have identified more than 12 species based on genetic analysis. These organisms also have been characterized serologically, with more than 200 pathogenic and 60 saprophytic serovars identified as of 2003.⁸⁷ These organisms are the cause of leptospirosis, a zoonotic disease of worldwide distribution. Growth of leptospirae in the laboratory requires specialized media and culture techniques, and cases of leptospirosis are usually diagnosed by serology.

Occupational Infections

Leptospirosis is a well-documented laboratory hazard. Approximately, 70 LAI and 10 deaths have been reported.^{4,26} Direct and indirect contact with fluids and tissues of experimentally or naturally infected mammals during handling, care, or necropsy are potential sources of infection.⁸⁸⁻⁹⁰ It is important to remember that rodents are natural carriers of leptospirae. Animals with chronic renal infection shed large numbers of leptospirae in the urine continuously or intermittently, for long periods of time. Rarely, infection may be transmitted by bites of infected animals.⁸⁸

Natural Modes of Infection

Human leptospirosis typically results from direct contact with infected animals, contaminated animal products, or contaminated water sources. Common routes of infection include abrasions, cuts in the skin or via the conjunctiva. Higher rates of infection observed in agricultural workers and other occupations associated with animal contact.

Laboratory Safety and Containment Recommendations

The organism may be present in urine, blood, and tissues of infected animals and humans. Ingestion, accidental parenteral inoculation, and direct and indirect contact of skin or mucous membranes, particularly the conjunctiva, with cultures or infected tissues or body fluids are the primary laboratory hazards. The importance of aerosol exposure is not known.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infective tissues, body fluids, and cultures. The housing and manipulation of infected animals should be performed at ABSL-2. Gloves should be worn to handle and necropsy infected animals and to handle infectious materials and cultures in the laboratory.

Special Issues

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

Listeria monocytogenes

Listeria monocytogenes is a gram-positive, non-spore-forming, aerobic bacillus; that is weakly beta-hemolytic on sheep blood agar and catalase-positive.⁹¹ The organism has been isolated from soil, animal feed (silage) and a wide range of human foods and food processing environments. It may also be isolated from symptomatic/asymptomatic animals (particularly ruminants) and humans.^{91,92} This organism is the causative agent of listeriosis, a food-borne disease of humans and animals.

Occupational Infections

Cutaneous listeriosis, characterized by pustular or papular lesions on the arms and hands, has been described in veterinarians and farmers.⁹³ Asymptomatic carriage has been reported in laboratorians.⁹⁴

Natural Modes of Infection

Most human cases of listeriosis result from eating contaminated foods, notably soft cheeses, ready-to-eat meat products (hot dogs, luncheon meats), paté and smoked fish/seafood.⁹⁵ Listeriosis can present in healthy adults with symptoms of fever and gastroenteritis, pregnant women and their fetuses, newborns, and persons with impaired immune function are at greatest risk of developing severe infections including sepsis, meningitis, and fetal demise. In pregnant women, *Listeria monocytogenes* infections occur most often in the third trimester and may precipitate labor. Transplacental transmission of *L. monocytogenes* poses a grave risk to the fetus.⁹²

Laboratory Safety and Containment Recommendations

Listeria monocytogenes may be found in feces, CSF, and blood, as well as numerous food and environmental samples.^{91,92,96,97} Naturally or experimentally infected animals are a source of exposure to laboratory workers, animal care

personnel and other animals. While ingestion is the most common route of exposure, *Listeria* can also cause eye and skin infections following direct contact with the organism.

BSL-2 practices, containment equipment, and facilities are recommended when working with clinical specimens and cultures known or suspected to contain the agent. Gloves and eye protection should be worn while handling infected or potentially infected materials. ABSL-2 practices, containment equipment and facilities are recommended for activities involving experimentally or naturally infected animals. Due to potential risks to the fetus, pregnant women should be advised of the risk of exposure to *L. monocytogenes*.

Special Issues

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

Mycobacterium leprae

Mycobacterium leprae is the causative agent of leprosy (Hansen disease). The organism has not been cultivated in laboratory medium but can be maintained in a metabolically active state for some period. Organisms are recovered from infected tissue and can be propagated in laboratory animals, specifically armadillos and the footpads of mice. The infectious dose in humans is unknown. Although naturally occurring leprosy or leprosy-like diseases have been reported in armadillos⁹⁸ and in NHP,^{99,100} humans are the only known important reservoir of this disease.

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

The infectious agent may be present in tissues and exudates from lesions of infected humans and experimentally or naturally infected animals. Direct contact of the skin and mucous membranes with infectious materials and accidental parenteral

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

BSL-2 practices, containment equipment, and facilities are recommended for all activities with known or potentially infectious materials from humans and animals. Extraordinary care should be taken to avoid accidental parenteral inoculation with contaminated sharp instruments. ABSL-2 practices, containment equipment, and facilities are recommended for animal studies utilizing rodents, armadillos, and NHP, because coughing with dissemination of infectious droplets does not occur in these species.

Special Issues

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

Mycobacterium tuberculosis complex

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

Occupational Infections

M. tuberculosis and *M. bovis* infections are a proven hazard to laboratory personnel as well as others who may be exposed to infectious aerosols in the laboratory, autopsy rooms, and other healthcare facilities.^{4,26,103-105} The incidence of tuberculosis in laboratory personnel working with *M. tuberculosis* has been reported to be three times higher than that of those not working with the agent.¹⁰⁶ Naturally or experimentally infected NHP are a proven source of human infection.¹⁰⁷ Experimentally infected guinea pigs or mice do not pose the same hazard because droplet nuclei are not produced by coughing in these species; however, litter from infected animal cages may become contaminated and serve as a source of infectious aerosols.

Natural Modes of Infection

M. tuberculosis is the etiologic agent of tuberculosis, a leading cause of morbidity and mortality worldwide. Persons infected with *M. tuberculosis* can develop active disease within months of infection or can remain latently infected and develop

disease later in life. The primary focus of infection is the lungs, but most other organs can be involved. HIV infection is a serious risk factor for development of active disease. Infectious aerosols produced by coughing spread tuberculosis. *M. bovis* is primarily found in animals but also can produce tuberculosis in humans. It is spread to humans, primarily children, by consumption of non-pasteurized milk and milk products, by handling of infected carcasses, and by inhalation. Human-to-human transmission via aerosols also is possible.

Laboratory Safety and Containment Recommendations

Tubercle bacilli may be present in sputum, gastric lavage fluids, CSF, urine, and in a variety of tissues. Exposure to laboratory-generated aerosols is the most important hazard encountered. Tubercle bacilli may survive in heat-fixed smears¹⁰⁸ and may be aerosolized in the preparation of frozen sections and during manipulation of liquid cultures. Because of the low infective dose of *M. tuberculosis* (i.e., ID₅₀ <10 bacilli), sputa and other clinical specimens from suspected or known cases of tuberculosis must be considered potentially infectious and handled with appropriate precautions. Accidental needle-sticks are also a recognized hazard.

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

BSL-3 practices, containment equipment, and facilities are required for laboratory activities in the propagation and manipulation of cultures of any of the subspecies of the *M. tuberculosis* complex and for animal studies using experimentally or naturally infected NHP. Animal studies using guinea pigs or mice can be conducted at ABSL-2.¹¹¹ BSL-3 practices should include the use of respiratory protection and the implementation of specific procedures and use of specialized equipment to prevent and contain aerosols. Disinfectants proven to be tuberculocidal should be used. See Appendix B for additional information.

Manipulation of small quantities of the attenuated vaccine strain *M. bovis* Bacillus Calmette-Guérin (BCG) can be performed at BSL-2 in laboratories that do not culture *M. tuberculosis* and do not have BSL-3 facilities. However, considerable care must be exercised to verify the identity of the strain and to ensure that cultures are not contaminated with virulent *M. tuberculosis* or other *M. bovis* strains. Selection of an appropriate tuberculocidal disinfectant is an important consideration for laboratories working with mycobacteria. See Appendix B for additional information.

Special Issues

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

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

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

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

More than 100 species of mycobacteria are recognized. These include both slowly growing and rapidly growing species. In the past, mycobacterial isolates that were not identified as *M. tuberculosis* complex were often called atypical mycobacteria, but these are now more commonly referred to as nontuberculous mycobacteria or mycobacteria other than tuberculosis. Many of the species are common environmental organisms, and approximately 25 of them are associated with infections in humans. A number of additional species are associated with infections in immunocompromised persons, especially HIV-infected individuals. All of these species are considered opportunistic pathogens in humans and none are considered communicable. Mycobacteria are frequently isolated from clinical samples but may not be associated with disease. The most common types of infections and causes are:

1. pulmonary disease with a clinical presentation resembling tuberculosis caused by *M. kansasii*, *M. avium*, and *M. intracellulare*;
2. lymphadenitis associated with *M. avium* and *M. scrofulaceum*;
3. disseminated infections in immunocompromised individuals caused by *M. avium*;
4. skin ulcers and soft tissue wound infections including Buruli ulcer caused by *M. ulcerans*, swimming pool granuloma caused by *M. marinum* associated with exposure to organisms in fresh and salt water and fish tanks, and tissue infections resulting from trauma, surgical procedures, or injection of contaminated materials caused by *M. fortuitum*, *M. chelonae*, and *M. abscessus*.

Occupational Infections

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

Natural Modes of Infection

Person-to-person transmission has not been demonstrated. Presumably, pulmonary infections are the result of inhalation of aerosolized bacilli, most likely from the surface of contaminated water. Mycobacteria are widely distributed in the environment and in animals. They are also common in potable water supplies, perhaps as the result of the formation of biofilms. The source of *M. avium* infections in immunocompromised persons has not been established.

Laboratory Safety and Containment Recommendations

Various species of mycobacteria may be present in sputa, exudates from lesions, tissues, and in environmental samples. Direct contact of skin or mucous membranes with infectious materials, ingestion, and accidental parenteral inoculation are the primary laboratory hazards associated with clinical materials and cultures. Aerosols created during the manipulation of broth cultures or tissue homogenates of these organisms also pose a potential infection hazard.

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

Special Issues

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

Neisseria gonorrhoeae

Neisseria gonorrhoeae is a gram-negative, oxidase-positive diplococcus associated with gonorrhea, a sexually transmitted disease of humans. The organism may be isolated from clinical specimens and cultivated in the laboratory using specialized growth media.¹¹²

Occupational Infections

Laboratory-associated gonococcal infections have been reported in the United States and elsewhere.¹¹³⁻¹¹⁶ These infections have presented as conjunctivitis, with either direct finger-to-eye contact or exposure to splashes of either liquid cultures or contaminated solutions proposed as the most likely means of transmission.

Natural Modes of Infection

Gonorrhea is a sexually transmitted disease of worldwide importance. The 2004 rate of reported infections for this disease in the United States was 112 per 100,000 population.¹¹⁷ The natural mode of infection is through direct contact with exudates from mucous membranes of infected individuals. This usually occurs by sexual activity, although newborns may also become infected during birth.¹¹²

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

Neisseria meningitidis

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

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

Occupational Infections

Recent studies of LAI and exposures have indicated that manipulating suspensions of *N. meningitidis* outside a BSC is associated with a high risk for contracting meningococcal disease.¹¹⁹ Investigations of potential laboratory-acquired cases of meningococcal diseases in the United States showed a many-fold higher attack rate for microbiologists compared to that of the United States general population age 30-59 years, and a case fatality rate of 50%, substantially higher than the 12-15% associated with disease among the general population. Almost all the microbiologists had manipulated sterile site isolates on an open laboratory bench.¹²⁰ While isolates obtained from respiratory sources are generally less pathogenic and consequently represent lower risk for microbiologists, rigorous protection from droplets or aerosols is mandated when microbiological procedures are performed on all *N. meningitidis* isolates, especially on those from sterile sites.

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

N. meningitidis may be present in pharyngeal exudates, CSF, blood, and saliva. Parenteral inoculation, droplet exposure of mucous membranes, infectious aerosol and ingestion are the primary hazards to laboratory personnel. Based on the mechanism of natural infection and the risk associated with handling of isolates on an open laboratory bench, exposure to droplets or aerosols of *N. meningitidis* is the most likely risk for infection in the laboratory.

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

If a BSC is unavailable, manipulation of these isolates should be minimized, primarily focused on serogroup identification using phenolized saline solution while wearing a laboratory coat, gloves, and safety glasses or full-face splash shield. BSL-3 practices and procedures are indicated for activities with a high potential for droplet or aerosol production and for activities involving production

quantities or high concentrations of infectious materials. Animal studies should be performed under ABSL-2 conditions.

Special Issues

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

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

Salmonella serotypes, other than S. Typhi

Salmonellae are gram-negative enteric bacteria associated with diarrheal illness in humans. They are motile oxidase-negative organisms that are easily cultivated on standard bacteriologic media, although enrichment and selective media may be required for isolation from clinical materials. Recent taxonomic studies have organized this genus into two species, *S. enterica* and *S. bongori*, containing more than 2500 antigenically distinct subtypes or serotypes.¹²³ *S. enterica* contains the vast majority of serotypes associated with human disease. *S. enterica* serotypes Typhimurium and Enteritidis (commonly designated *S. Typhimurium* and *S. Enteritidis*) are the serotypes most frequently encountered in the United States. This summary statement covers all pathogenic serotypes except *S. Typhi*.

Occupational Infections

Salmonellosis is a documented hazard to laboratory personnel.^{4,26,124-125} Primary reservoir hosts include a broad spectrum of domestic and wild animals, including birds, mammals, and reptiles, all of which may serve as a source of infection to laboratory personnel. Case reports of laboratory-acquired infections indicate a presentation of symptoms (fever, severe diarrhea, abdominal cramping) similar to those of naturally-acquired infections, although one case also developed erythema nodosum and reactive arthritis.^{126,127}

Natural Modes of Infection

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

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

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

Salmonella Typhi

Recent taxonomic studies have organized the genus *Salmonella* into two species, *S. enterica* and *S. bongori*, containing more than 2500 antigenically distinct subtypes or serotypes.¹²³ *S. enterica* contains the vast majority of serotypes associated with human disease. *S. enterica* serotype Typhi, commonly designated *S. Typhi*, is the causative agent of typhoid fever. *S. Typhi* is a motile gram-negative enteric bacterium that is easily cultivated on standard bacteriologic media, although enrichment and selective media may be required for isolation of this organism from clinical materials.

Occupational Infections

Typhoid fever is a demonstrated hazard to laboratory personnel.^{4,129,130} Ingestion and less frequently, parenteral inoculation are the most significant modes of transmission in the laboratory. Secondary transmission to other individuals outside of the laboratory is also a concern.¹³¹ Laboratory-acquired *S. Typhi* infections usually present with symptoms of septicemia, headache, abdominal pain, and high fever.¹²⁹

Natural Modes of Infection

Typhoid fever is a serious, potentially lethal bloodstream infection of worldwide distribution. Humans are the sole reservoir and asymptomatic carriers may occur. The infectious dose is low (<103 organisms) and the incubation period may vary from one to six weeks, depending upon the dose of the organism. The natural mode of transmission is by ingestion of food or water contaminated by feces or urine of patients or asymptomatic carriers.¹²³

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

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

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

Shiga toxin (Verocytotoxin)-producing Escherichia coli

Escherichia coli is one of five species in the gram-negative genus *Escherichia*. This organism is a common inhabitant of the bowel flora of healthy humans and other mammals and is one of the most intensively studied prokaryotes. An extensive serotyping system has been developed for *E. coli* based on the O (somatic) and H (flagellar) antigens expressed by these organisms. Certain pathogenic clones of *E. coli* may cause urinary tract infections, bacteremia, meningitis, and diarrheal disease in humans, and these clones are associated with specific serotypes.

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

Occupational Infections

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

Natural Modes of Infection

Cattle represent the most common natural reservoir of Shiga-toxin producing *E. coli*. Transmission usually occurs by ingestion of contaminated food, including raw milk, fruits, vegetables, and particularly ground beef. Human-to-human transmission has been observed in families, day care centers, and custodial institutions. Water-borne transmission has been reported from outbreaks

associated with swimming in a crowded lake and drinking unchlorinated municipal water.¹³⁹ In a small proportion of patients (usually children) infected with these organisms, the disease progresses to hemolytic uremic syndrome or death.

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

Shigella

The genus *Shigella* is composed of nonmotile gram-negative bacteria in the family Enterobacteriaceae. There are four subgroups that have been historically treated as separate species, even though more recent genetic analysis indicates that they are members of the same species. These include subgroup A (*Shigella dysenteriae*), subgroup B (*S. flexneri*), subgroup C (*S. boydii*), and subgroup D (*S. sonnei*). Members of the genus *Shigella* have been recognized since the late 19th century as causative agents of bacillary dysentery, or shigellosis.¹²³

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

Special Issues

Vaccines Vaccines are currently not available for use in humans.

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

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

Treponema pallidum

Treponema pallidum is a species of extremely fastidious spirochetes that die readily upon desiccation or exposure to atmospheric levels of oxygen, and have not been cultured continuously *in vitro*.¹⁴⁵ *T. pallidum* cells have lipid-rich outer membranes and are highly susceptible to disinfection with common alcohols (i.e., 70% isopropanol). This species contains three subspecies including *T. pallidum* spp. *pallidum* (associated with venereal syphilis), *T. pallidum* spp. *endemicum* (associated with endemic syphilis), and *T. pallidum* spp. *pertenue* (associated with Yaws). These organisms are obligate human pathogens.

Occupational Infections

T. pallidum is a documented hazard to laboratory personnel. Pike lists 20 cases of LAI.⁴ Syphilis has been transmitted to personnel working with a concentrated suspension of *T. pallidum* obtained from an experimental rabbit orchitis.¹⁴⁶ *T. pallidum* is present in the circulation during primary and secondary syphilis. The ID₅₀ of *T. pallidum* needed to infect rabbits by subcutaneous injection has been reported to be as low as 23 organisms.¹⁴⁷ The concentration of *T. pallidum* in patients' blood during early syphilis, however, has not been determined. No cases of laboratory animal-associated infections are reported; however, rabbit-adapted *T. pallidum* (Nichols strain and possibly others) retains virulence for humans.

Natural Modes of Infection

Humans are the only known natural reservoir of *T. pallidum* and transmission occurs via direct sexual contact (venereal syphilis), direct skin contact (Yaws), or direct mucous contact (endemic syphilis). Venereal syphilis is a sexually transmitted disease that occurs in many areas of the world, whereas Yaws occurs in tropical areas of Africa, South America, the Caribbean, and Indonesia. Endemic syphilis is limited to arid areas of Africa and the Middle East.¹⁴⁵

Laboratory Safety and Containment Recommendations

The agent may be present in materials collected from cutaneous and mucosal lesions and in blood. Accidental parenteral inoculation, contact with mucous membranes or broken skin with infectious clinical materials are the primary hazards to laboratory personnel.

BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of blood or other clinical samples from humans or infected rabbits. Gloves should be worn when there is a likelihood

of direct skin contact with infective materials. Periodic serological monitoring should be considered in personnel regularly working with these materials. ABSL-2 practices, containment equipment, and facilities are recommended for work with infected animals.

Special Issues

Vaccines Vaccines are currently not available for use in humans.

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

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

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

Occupational Infections

Rare cases of bacterial enteritis due to LAI with either *V. cholerae* or *V. parahaemolyticus* have been reported from around the world.⁴ Naturally and experimentally infected animals¹⁴⁹ and shellfish^{150,151} are potential sources for such illnesses.

Natural Modes of Infection

The most common natural mode of infection is the ingestion of contaminated food or water. The human oral infecting dose of *V. cholerae* in healthy non-achlorhydric individuals is approximately 10^6 - 10^{11} colony forming units,¹⁵² while that of *V. parahaemolyticus* ranges from 10^5 - 10^7 cells.¹⁵³ The importance of aerosol exposure is unknown although it has been implicated in at least one instance.¹⁴⁹ The risk of infection following oral exposure is increased in persons with abnormal gastrointestinal physiology including individuals on antacids, with achlorhydria, or with partial or complete gastrectomies.¹⁵⁴

Laboratory Safety and Containment Recommendations

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

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

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

Special Issues

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

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

Yersinia pestis

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

Occupational Infections

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

Natural Modes of Infection

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

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

Laboratory Safety and Containment Recommendations

The agent has been isolated, in order of frequency of recovery, from bubo aspirate, blood, liver, spleen, sputum, lung, bone marrow, CSF, and infrequently from feces and urine, depending on the clinical form and stage of the disease. Primary hazards to laboratory personnel include direct contact with cultures and infectious materials from humans or animal hosts and inhalation of infectious aerosols or droplets generated during their manipulation. Laboratory and field personnel should be counseled on methods to avoid fleabites and accidental autoinoculation when handling potentially infected live or dead animals.

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

Special Issues

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

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

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Section VIII-B: Fungal Agents

Blastomyces dermatitidis

Blastomyces dermatitidis is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia) that are the infectious particles; these convert to large budding yeasts under the appropriate culture conditions *in vitro* at 37°C and in the parasitic phase *in vivo* in warm-blooded animals. The sexual stage is an Ascomycete with infectious ascospores.

Occupational Infections

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

Natural Modes of Infection

The fungus has been reported from multiple geographically separated countries, but is best known as a fungus endemic to North America and in association with plant material in the environment. Infections are not communicable, but require common exposure from a point source. Although presumed to dwell within the soil of endemic areas, *B. dermatitidis* is extremely difficult to isolate from soil. Outbreaks associated with the exposure of people to decaying wood have been reported.¹¹

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

Coccidioides immitis* and *Coccidioides posadasii

Coccidioides spp. is endemic to lower sonoran deserts of the western hemisphere including northern Mexico, southern Arizona, central and southern California, and west Texas. The original species (*C. immitis*) has been divided into *C. immitis* and *C. posadasii*.¹² These species are dimorphic fungal pathogens existing in nature and in laboratory cultures at room temperature as filamentous molds with asexual spores (single-cell arthroconidia three to five microns in size) that are the infectious particles that convert to spherules under the appropriate culture conditions *in vitro* at 37°C and *in vivo* in warm-blooded animals.

Occupational Infections

Laboratory-associated coccidioidomycosis is a documented hazard of working with sporulating cultures of *Coccidioides* spp.¹³⁻¹⁵ Occupational exposure has also been associated in endemic regions with archeology¹⁶ and high dust exposure.¹⁷ Attack rates for laboratory and occupational exposure are higher than for ambient exposure when large numbers of spores are inhaled. Smith reported that 28 of 31 (90%) laboratory-associated infections in his institution resulted in clinical disease, whereas more than half of infections acquired in nature were asymptomatic.¹⁸ Risk of respiratory infection from exposure to infected tissue or aerosols of infected secretions is very low. Accidental percutaneous inoculation has typically resulted in local granuloma formation.¹⁹

Natural Modes of Infection

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

The majority of ambient infections is subclinical and results in life-long protection from subsequent exposures. The incubation period is one to three weeks and manifests as a community-acquired pneumonia with immunologically mediated fatigue, skin rashes, and joint pain. One of the synonyms for coccidioidomycosis is desert rheumatism. A small proportion of infections is complicated by hematogenous dissemination from the lungs to other organs, most frequently skin, the skeleton, and the meninges. Disseminated infection is

much more likely in persons with cellular immunodeficiencies (AIDS, organ transplant recipient, lymphoma).

Laboratory Safety and Containment Recommendations

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

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

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

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

Special Issues

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

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

Cryptococcus Neoformans

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

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

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

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

Histoplasma capsulatum

Histoplasma capsulatum is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia); these are the infectious particles that convert to small budding yeasts under the appropriate culture conditions *in vitro* at 37°C

and in the parasitic phase *in vivo*. The sexual stage is an Ascomycete with infectious ascospores.

Occupational Infections

Laboratory-associated histoplasmosis is a documented hazard in facilities conducting diagnostic or investigative work.²⁴⁻²⁷ Pulmonary infections have resulted from handling mold form cultures.^{28,29} Local infection has resulted from skin puncture during autopsy of an infected human,³⁰ from accidental needle inoculation of a viable culture,³¹ and from spray from a needle into the eye.³² Collecting and processing soil samples from endemic areas has caused pulmonary infections in laboratory workers.³³ Conidia are resistant to drying and may remain viable for long periods of time. The small size of the infective conidia (less than 5 microns) is conducive to airborne dispersal and intrapulmonary retention. Work with experimental animals suggests that hyphal fragments are capable of serving as viable inocula.²⁴

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with starling and bat feces. It has been isolated from soil, often in river valleys, between latitudes 45°N and 45°S. Histoplasmosis is naturally acquired by the inhalation of infectious particles, usually microconidia.²⁴ Infections are not transmissible from person-to-person, but require common exposure to a point source.

Laboratory Safety and Containment Recommendations

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

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, animal tissues and mold cultures, identifying cultures in routine diagnostic laboratories, and for inoculating experimental animals, regardless of route. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

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

Special Issues

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

Sporothrix schenckii

Sporothrix schenckii is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia); these are the infectious particles that convert to small budding yeasts in the parasitic phase *in vivo*. The sexual stage is unknown.

Occupational Infections

Most cases of sporotrichosis are reported sporadically following accidental inoculation with contaminated material. Large outbreaks have been documented in persons occupationally or recreationally exposed to soil or plant material containing the fungus. However, *S. schenckii* has caused a substantial number of local skin or eye infections in laboratory personnel.³⁴ Most occupational cases have been associated with accidents and have involved splashing culture material into the eye,^{35,36} scratching,³⁷ or injecting³⁸ infected material into the skin or being bitten by an experimentally infected animal.^{39,40} Skin infections in the absence of trauma have resulted also from handling cultures⁴¹⁻⁴³ or necropsy of animals⁴⁴ without any apparent trauma.

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

Although localized skin and eye infections have occurred in an occupational setting, no pulmonary infections have been reported as a result from laboratory exposure. It should be noted that serious disseminated infections have been reported in immunocompromised persons.⁴⁵

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

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

Special Issues

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

Dermatophytes (Epidermophyton, Microsporum, and Trichophyton)

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

Occupational Infections

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

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

Miscellaneous Molds

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

Occupational Infections

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

Natural Modes of Infection

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

Laboratory Safety and Containment Recommendations

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

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

Special Issues

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

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Section VIII-C: Parasitic Agents

General Issues

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

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

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

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

Blood and Tissue Protozoal Parasites

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

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

Occupational Infections

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

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

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

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

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

Natural Modes of Infection

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

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

Laboratory Safety and Containment Recommendations

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

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

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

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

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

Special Issues

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

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

Intestinal Protozoal Parasites

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

Occupational Infections

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

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

Natural Modes of Infection

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

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

Laboratory Safety and Containment Recommendations

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

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

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

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

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

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

Special Issues

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

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

Trematode Parasites

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

Occupational Infections

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

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

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

Natural Modes of Infection

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

Schistosoma mansoni is widely distributed in Africa, South America, and the Caribbean; the prevalence of infection has been rapidly changing in some areas. Infection occurs when persons are exposed to free-swimming cercariae in contaminated bodies of water; cercariae can penetrate intact skin. The natural snail hosts capable of supporting development of *S. mansoni* are various species of *Biomphalaria*.

Laboratory Safety and Containment Recommendations

Infective stages of *F. hepatica* (metacercariae) and *S. mansoni* (cercariae) may be found, respectively, encysted on aquatic plants or in the water in laboratory aquaria used to maintain snail intermediate hosts. Ingestion of fluke metacercariae and skin penetration by schistosome cercariae are the primary laboratory hazards. Dissection or crushing of schistosome-infected snails may also result in exposure of skin or mucous membrane to cercariae-containing droplets. Additionally, metacercariae may be inadvertently transferred from hand to mouth by fingers or gloves, following contact with contaminated aquatic vegetation or aquaria.

All reported cases of laboratory-acquired schistosomiasis have been caused by *S. mansoni*, which probably reflects the fact that many more laboratories work with *S. mansoni* than with other *Schistosoma* spp. However, accidental infection with *S. haematobium*, *S. japonicum*, and *S. mekongi* could easily occur in the same manner as described for *S. mansoni*.

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

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

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

Special Issues

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

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

Cestode Parasites

Cestode parasites of potential risk for laboratorians include *Echinococcus* spp., *Hymenolepis nana*, and *Taenia solium*. Echinococcosis is an infection caused by cestodes in the genus *Echinococcus*; *E. granulosus* causes cystic echinococcosis, *E. multilocularis* causes alveolar echinococcosis, and *E. vogeli* and *E. oligarthrus* cause polycystic echinococcosis. Humans serve as intermediate hosts and harbor the metacestode or larval stage, which produces a hydatid cyst. *Hymenolepis nana*, the dwarf tapeworm, is cosmopolitan in distribution and produces hymenolepiasis, or intestinal infection with the adult tapeworm. *Taenia solium*, the pork tapeworm, causes both taeniasis (infection of the intestinal tract with the adult worm), and cysticercosis (infection of subcutaneous, intermuscular, and central nervous system with the metacestode stage or cysticercus).

Occupational Infections

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

Natural Modes of Infection

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

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

Laboratory Safety and Containment Recommendations

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

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

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

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

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

Nematode Parasites

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

Occupational Infections

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

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

Natural Modes of Infection

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

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

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

Enterobius vermicularis occurs worldwide, although infection tends to be more common in school-age children than adults, and in temperate than tropical regions. Pinworm infection is acquired by ingestion of infective eggs, most often on contaminated fingers following scratching of the perianal skin. Eggs passed by female worms are not immediately infective, but only require several hours' incubation to become fully infectious. Infection with this worm is relatively short (60 days on average), and reinfection is required to maintain an infection.

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

Laboratory Safety and Containment Recommendations

Eggs and larvae of most nematodes are not infective in freshly passed feces; development to the infective stages may require from one day to several weeks. Ingestion of the infective eggs or skin penetration by infective larvae are the primary hazards to laboratory staff and animal care personnel. Development of hypersensitivity is common in laboratory personnel with frequent exposure to aerosolized antigens of ascarids.

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

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