TITLE: Biosafety Plan (Unit Specific)

SOURCE: Los Angeles County Department of Public Health Laboratory - Bioterrorism Response Unit (BTRU)

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A. INTRODUCTION

1. This document was developed to ensure compliance with the site-specific requirements of the Select Agent Final Rule as enumerated in § 9 CFR 121.12, § 6, 7 CFR 331.12, § 43 CFR 73.12.
2. This is the Bioterrorism Response Unit specific biosafety plan for use of *Brucella* species, exempt quantities of ricin toxin, patient specimens and environmental samples including credible threat samples submitted by law enforcement for purposes of confirmatory testing of select agents and toxins. This plan also includes other select agents such as those now designated as Tier 1 that may be encountered during LRN testing procedures. These agents will be properly transferred or destroyed as appropriate after testing.
3. This plan is supplemental to the departmental Biosafety Plan within the *Injury and Illness Prevention Program Manual (IIPP)* and complements the *Bioterrorism Response Unit Incident Response Plan (LACPHL-BTRU-POL-010).*

B. DEFINITIONS

1. Responsible Official (R.O.) means the individual designated by the entity with authority and control to ensure compliance with the regulations in 42 CFR part 73. (1)
2. Alternate Responsible Official (A.R.O.) acts in the capacity of the R.O. in his/her absence. (1)
3. Principal Investigators (P.I.) the one individual who is designated by the entity to direct a project or program and who is responsible to the entity for the scientific and technical direction of the project or program. (1)

C. ROLES AND RESPONSIBILITIES

1. **Principal Investigator**: It is the primary responsibility of the P.I. for the implementation of the select agent program.
2. Establish incident response procedures specific to situations related to risks, associated with select agents and toxins, personnel, the environment, and the community, this includes the security of select agents and toxins from theft, natural and manmade disasters.
3. Provide for on-going emergency training for staff on an annual basis.
4. Laboratory safety and security of select agents
5. Coordinate emergency response activities with local law enforcement and emergency first-responders such as HazMat teams and EMTs.
6. Communicate with Responsible Official (R.O.) the day-to-day status of the BTRU and immediately report all “incidents” that arise within the BTRU to the R.O.
7. Comply with all reporting requirements delineated in both state and federal regulations.
8. Perform quarterly inventory of select agents for submission, review and acceptance by the Emergency Response Coordinator, Alternate Responsible Official, or Responsible Official.

9. Report the identification and final disposition of any select agent or toxin contained in a specimen presented for diagnosis or verification and inter-entity transfers
10. Oversight of the select agent program is performed by the **Responsible Official**, the **Alternate Responsible Official** and the **Biosafety Officer**.
    a. Develop exercises and drills, conducted at least annually, to test and evaluate the effectiveness of the *Incident Response Plan* and readiness of staff.
    b. Prepare *After Action Reports* for the exercises and drills and revise plans as warranted.
    c. Record keeping as it relates to Security Risk Assessment of staff with access to select agents.
    d. Annual Program Review. The **Responsible Official** or **Alternate Responsible Official** will audit the incident response program on an annual basis. This review will include drills and exercises to ensure the effectiveness of the incident response plan. Based on the outcome of drills, exercises or reported incidents, this incident response plan may be updated.
    e. Where required, the Responsible Official will report incidents to CDC.

D. ROUTES OF INFECTION AND LABORATORY ACTIVITIES (GENERAL)

Inhalation: Activities that generate aerosols

1. **Manipulating needles and syringe**
    a. Expelling air from tubes or bottles
    b. Withdrawing needles from stoppers
    c. Separating needles from syringes
2. **Manipulating inoculation needles or loops**
    a. Flaming loops
    b. Cooling loops in culture media
    c. Subculturing and streaking culture media
3. **Manipulating pipettes**
    a. Mixing microbial suspensions
    b. Spilling microbial suspensions on hard surfaces
4. **Manipulating specimens and cultures**
    a. Centrifugation
    b. Mixing, blending, grinding, shaking, sonication, and vortexing of specimens or cultures
    c. Pouring or decanting fluids
    d. Removing caps or swabs from culture containers
    e. Spilling infectious material
    f. Filtering specimens under vacuum

Ingestion: Activities related to oral transmission

1. **Pipetting by mouth**
2. **Splashing contaminated material into the mouth**
3. **Placing contaminated material or fingers in the mouth**
4. **Eating, drinking, using lipstick, and smoking in the workplace**

Inoculation: Activities related to direct intravenous and subcutaneous transmission

1. **Manipulating needles and syringes**
2. **Handling broken glass, scalpels, and other sharp objects**

Inoculation: Activities related to contaminated skin and mucous membranes

1. **Splashing or spilling material into eyes, mouth, and nose and onto skin**
2. **Exposing nonintact skin to contaminated material**
3. **Working on contaminated surfaces**
4. **Handling contaminated equipment**
5. **Inappropriate handling of loops, inoculating needles, or swabs containing specimen or culture material**

E. SAFE WORK PRACTICES

All laboratory personnel must follow standard precautions guidelines. Specimens received in the laboratory should be worked with as if they contain potentially infectious material. The implementation of safe work practices is essential for the prevention of laboratory-associated infections.

Handling of Specimens

A. Gloves and gowns

1. Wear gloves and gowns (impervious to liquids) at all times when handling and processing patient specimens, decontaminating instruments and countertops, and cleaning spills.
2. Bandage open cuts and scratches on hands and then wear gloves.
3. Wash hands immediately after gloves are removed, after a task that involves heavily contaminated matter, and before leaving the laboratory.

B. Specimen transport

1. Specimens and samples need to be transported in leakproof containers with the biohazard symbol affixed.
2. *Do not* accept grossly soiled or contaminated specimens. Notify an individual responsible for submitting such a specimen immediately, and follow the laboratory's specimen rejection policy.

C. Needles and syringes

1. Use needle-locking syringes or plastic disposable syringe-needle units.
2. Never recap or bend needles or remove them from syringes.
3. *Do not* accept specimens received in syringes with needles attached. Notify the individual responsible for submitting such a specimen immediately and follow the laboratory's specimen rejection policy.
4. Discard in a puncture-resistant container that has the biohazard symbol affixed.
5. Use needle-less systems for blood samples if available.
6. Use mechanical devices or one-handed techniques when handling sharp objects.
7. Secure bottles before inserting needles into the bottles (e.g., place bottles in support racks).

D. Tubes

1. Carry tubes in racks.
2. Use plastic tubes when possible.
3. Uncap tubes behind a clear plastic shield or inside a BSC to contain splashes or sprays (e.g., when removing tops from vacuum tubes).

E. Centrifuges

1. Centrifuge tubes must be intact and properly balanced when centrifuged.
2. Centrifuge tubes used in the BSL-3 suite must be enclosed in sealed safety cups.
3. Use aerosol-free centrifuges when possible.
4. Centrifuge safety cups must be opened in a biological safety cabinet (BSC) after centrifugation.
5. Decontaminate the entire rotor prior to removal from the BSC.
6. *Do not* place tabletop centrifuges in a BSC because air turbulence within the cabinet can allow aerosols to escape.

F. Hand washing

1. Perform frequent hand washing after removing gloves, before leaving the laboratory, and before eating, drinking, or applying cosmetics.
2. Use nonirritating soap for routine washing.
3. Use antiseptic soap or an alcohol planchet followed by thorough hand washing for accidental skin contamination.

G. Eye Protection

1. Wear only disposable face shields
2. Goggle are not to be worn during testing (see BTRU-POL-00-30)

Processing of Specimens

NOTE: *See* each individual LRN specific procedure for specifics; these are meant as general guidelines.

A. Process all specimens in a BSC
B. Properly label per protocol.

C. Use disposable bacteriological to wire needles and loops which require no heating include plastic disposable loops and spreaders for streaking plates and spreading material onto slides.
D. Mix or transfer liquids by using disposable plastic pipettes and a rubber bulb.
E. Alternatively, use mechanical pipetting devices.
F. Cover tubes when mixing, blending, vortexing, etc. (e.g., cap tubes or cover them with Parafilm).
G. Work over an absorbent covering or disinfectant-moistened mat (e.g., phenolic-compound-soaked pad).
H. Plan tasks to minimize exposure to known hazards.

- Follow standard precautions when performing non-culture techniques (e.g., antibody, antigen, and molecular detection methods).

Removal of Specimens from the Biological Safety Cabinet

Preparing Agent Vessels for Removal from BSC

a. All materials removed from the BSC must be labeled properly (SOP BTRU-G005)

b. Plates must be taped together in stacks, with tape on both sides of stack, making sure the lid on the last plate in the stack is firmly taped in place. The stacks may not be more than 8 plates tall. Individual plates must be taped to prevent accidental exposure.

c. Materials for storage at -70°C or below must be placed in screw-cap cryovials that can withstand ultra-low temperatures. Vials containing liquid culture must allow space for expansion during the freezing process. All materials must be appropriately labeled in a manner that will ensure legibility throughout storage at ultra-low temperatures.

d. Liquid cultures must be contained in plastic vessels (erylenmyer flasks) with screw-top caps. Caps must be tightened prior to movement from the BSC. If cultures require aeration during growth, caps can be loosed once the cultures are placed in the incubator.

e. Tube cultures must be in plastic tubes with screw-tops. Caps must be tightened prior to removal from the BSC. Tubes must be placed in a rack for transport. If the tubes are intended for incubation, caps may be loosed after being placed in incubator. If tubes are for shipment they must be sealed with parafilm.

f. Samples for PCR must be placed in vials having screw-top lids with o-rings. Vials must be placed in racks prior to removal from the BSC.

Removal of Vessels from the BSC

a. All primary vessels must be decontaminated prior to removal from the BSC. (see APPENDIX)

b. Vessels containing an agent must be contained in a secondary transport container that is leak-proof and can be autoclaved. The secondary container must be decontaminated prior to removal from the BSC. (see APPENDIX)

c. All vessels containing a select agent (confirmed or suspected) must be properly labeled and removed to a secure area such as a locked freezer, incubator, or refrigerator.

Movement of Materials to the BSC:

a. Agent must be transported to the BSC in a transport box or other sealed secondary container.

b. Frozen agents of <1.0 ml stored in screw-cap containers should be moved from the freezer to the BSC within a secondary container.

Housekeeping and Miscellaneous Safe Practices

A. General

1. Avoid or minimize activities associated with the transmission of infectious agents.
2. Designate clean and contaminated work areas.
   a. Wear gloves in contaminated areas.
   b. Clean and disinfect all surfaces after spills and at the end of each work shift.
3. Keep all work areas neat and uncluttered.
   a. *Do not* store personal items in the work areas.
   b. *Do not* store large quantities of disposable items in the work areas.
4. Remove coats and gowns before leaving the laboratory. Place contaminated laundry (e.g., reusable scrubs) in brown paper bags for autoclaving by the institution and then laundered off site.
5. Dispose of all contaminated material in containers for treatment (e.g., by autoclaving).

H. BIOSAFETY LEVELS

BSLs are guidelines that describe appropriate containment equipment, facilities, and procedures for use by laboratory workers. The BSLs range from BSL 1 to BSL 4. Each BSL is based on the increased risk associated with the pathogenicity of the microorganisms encountered. When working with highly infectious agents for which the risk of aerosol transmission is greater (e.g., *Brucella* species, *Francisella* species, follow BSL 3 practices. Information on BSL recommendations for specific microorganisms can be found in the agent specific section.

BSL-1

BSL 1 is recommended for work with microorganisms not known to cause disease in healthy adults (e.g., *Bacillus subtilis*).

A. Restrict access to authorized personnel.
B. Make sinks for hand washing readily accessible.
C. Make eyewash stations readily accessible.
D. Make appropriate PPE available and ensure use.
E. Ensure that laboratory bench tops are impervious to liquids and resistant to chemicals.
F. Ensure that laboratory surfaces and equipment are easily cleaned and disinfected and that these procedures are done on a regular basis or whenever the surfaces or equipment is contaminated.
G. Decontaminate solid waste within the laboratory (e.g., by autoclaving),or package the waste to be transported off-site.

BSL-2

BSL 2 is recommended for microorganisms associated with human disease but not transmitted by aerosols (e.g., *Salmonella* species).

A. Follow BSL 1 practices plus the following.
B. Display universal biohazard signs outside of the laboratory
C. Perform specimen processing in a biological safety cabinet (BSC)
D. Perform centrifugation of specimens by using centrifuge safety cups
E. Ensure that an autoclave or other decontamination equipment is available and used for treatment of infectious waste
F. Use the appropriate PPE (e.g., gowns, gloves, and facial barriers).
G. Place all sharps carefully in conveniently located, puncture-resistant containers.
H. Trained personnel must observe good microbiological practices and techniques.

BSL-3

BSL 3 is recommended for hazardous microorganisms primarily transmitted by aerosols (e.g., *Brucella sp. and Francisella sp.*).

A. Follow BSL 1 and BSL 2 practices plus the following.
B. Control access to the laboratory.
C. Perform all manipulations of cultures and clinical material in a BSC (class II).
D. Maintain a negative-pressure airflow in the laboratory.
E. Include double doors and an anteroom in the laboratory design.
F. Discharge HEPA-filtered exhaust air from BSCs outside the facility.
G. Use all appropriate PPE and containment devices.
H. Use HEPA-filtered respirators or masks when aerosols may be generated.
I. Collect baseline serum samples from all personnel for serological determination of immune status.
J. Post specific agent signs on doors to the ante room and 149 when agents are being worked with to minimize traffic and to alert other personnel.

F. BIOLOGICAL SAFETY CABINET

The biological safety cabinet (BSC) is the most important primary containment equipment in the clinical microbiology laboratory. Class II A laminar-flow cabinets are used for BSL 2 and 3 practices. Air is drawn into the cabinet by negative air pressure and passes through a HEPA filter. The air flows in a vertical sheet that serves as a barrier between the outside and the inside of the cabinet. The cabinet exhaust air is also passed through HEPA filters. Aerosols are contained within the BSC, and the work area is protected from outside contamination when the cabinet is operating under the manufacturer's recommended conditions.

Procedures

A. Pre-operation

1. If off, turn on blowers for at least 10 min before QC checks and specimen processing. Cabinets located in the BSL-3 suite are left on continuously.
2. Ensure the viewscreen is at the correct operating height (8 in.) as when certified.
3. Turn off UV lights (if present).
4. Turn on a fluorescent light.
5. Perform daily QC as outlined below.
6. Clean the cabinet working surfaces with appropriate disinfectant.

B. Operation

1. Avoid outside sources of air currents (e.g., personnel walking by, doors being opened or closed, etc.).
2. Wear appropriate PPE for the task.
3. Place all items that will be used in a planned activity inside the cabinet prior to starting work.
    a. Segregate clean from contaminated materials.
    b. Place the minimum number of large devices and supplies inside the cabinet.
        (1) *Do not* block intake or rear grills.
        (2) *Do not* place sterile material or specimens near the sides, front, or back of the cabinet.
        (3) *Do not* place or tape paper notes or procedures on the window.
        (4) *Do not* use a flame in the cabinet.
        (5) *Do not* operate centrifuges in the cabinet.
4. Plan work flow to minimize movements.
    a. Work at least 6 in. inside the front grill intake.
    b. Avoid rapid arm movement in and out of the cabinet while a procedure is in progress.
    c. If necessary, remove outer gloves and discard then within the BSC, then slowly move arms out of the cabinet.

C. Post-operation

1. Allow the cabinet to continue running for 15 to 20 min after work is completed and before removing materials.
2. Allow the cabinet to continue running for at least 3 h after processing.
3. Place contaminated materials in covered containers or closed bags, or immerse them in disinfectant, before removal from the cabinet.
4. Disinfect the surfaces of any contaminated materials before removal from the cabinet.
5. Clean up spills by following the steps outlined in the procedure
6. Clean interior surfaces with disinfectant (e.g., 70% ethanol)
7. *Do not* shut down cabinets that function 24 h a day, such as those located in the BSL-3 suite unless a malfunction occurs or routine maintenance is required.

Quality Control

A. Daily
   1. Disinfect all cabinet surfaces while cabinet is running.
   2. Check air velocity (recommended for BSCs not equipped with velocity gauges and airflow indicators in the work area).
      a. Place a Vaneometer at the opening of the cabinet toward the work space area but not on the airflow grill.
      b. Level the Vaneometer and record face airflow velocity.
         (1) the air velocity should be greater than 100 ft/min.
         (2) *Do not* use the BSC if velocity remains less than 100 ft/min. Contact a technical service consultant.
   3. Check the blower function.
      a. Record the manometer gauge reading (if provided).
      b. Perform a visible-smoke test.
         (1) Pass a smoke source (e.g., dry ice in water or smoke stick; from one end of the cabinet opening to the other.
         (2) The smoke should show a smooth downward flow with no deadspots or reflux.
         (3) Record results.
      c. Observe airflow check strips (optional).
         (1) Strips should be drawn toward the work space area.
         (2) Record results.

B. Weekly
   1. Clean UV lights (if present and used) with 70% ethanol.

C. Monthly
   1. Clean the gutter area with disinfectant.

D. Semiannually
   1. Have BSCs re-certified by certified personnel.
   2. Have certified personnel measure UV light output (if present and used).

E. Complete and initial the Biological Safety Cabinet Quality Control and Maintenance Record worksheet after each task is completed.

Limitation of the Procedure

A.BSCs should not be used as fume hoods.

1.Toxic, radioactive, or flammable vapors or gases are not removed by HEPA filters.

2.Potentially hazardous amounts of volatile material may build up in class II-type cabinets.

3.Exhausted vapors can be vented into the laboratory air.

B.The use of gloves when performing routine manipulations should not substitute for proper hand washing practices. Wash hands with soap and water between tasks and when heavily soiled with clinical material.

C. BIOHAZARDOUS SPILLS

Materials

A. Disinfectants

1. 10% Household bleach

B. Equipment and supplies

1. Paper towels
2. PPE
3. Autoclavable squeegee and dust pan
4. Tongs, forceps, or hemostats
5. Autoclavable plastic bags
6. Biohazardous-waste container

Procedure

A. Cleanup of major spills (possible aerosol formation.

1. Evacuate the area or room, taking care not to breathe in aerosolized material.
2. Alert personnel in the laboratory to evacuate the area.
3. Close the doors to the affected area.
4. After 30 min, when aerosols have settled, enter the area to clean.
   a. Cleanup should be performed by the individual who committed the spill or by assigned personnel.
   b. PPE should include gloves (e.g.,heavy weight utility), disposable booties or water-impermeable shoe covers/boots, long-sleeved gowns, and masks.
   c. For high-risk agents, a full-faced respirator or HEPA-filtered mask should also be used.
5. Remove and discard any broken glass or other objects.
   a. *Do not* allow contact with hands.

   b. Use rigid cardboard or squeegee and dustpan, tongs, forceps, or hemostats.
   c. Discard these items into a plastic biohazardous-waste container.
6. Cover the spill with disposable absorbent material (e.g., paper towels).
7. After absorption of liquid, discard all contaminated material in a biohazardous-waste container.
8. Carefully clean the spill site of any visible material, from the edges of the spill into the center, with an aqueous detergent solution.
9. Pour fresh disinfectant on the spill site or wipe down the site with disinfectant-soaked disposable towels.
10. Allow disinfectant to remain on the site for 20 min.
11. Absorb disinfectant solution with disposable material or allow the disinfectant to dry.
12. Rinse the spill site with water, and air dry to prevent slipping.
13. Discard all paper towels, gloves, and other disposable items into an autoclavable plastic bag or biohazardous-waste container.
14. Place gowns in a container for autoclaving after the cleanup process is completed.
15. Wash hands with soap and water.

B. Cleanup of minor spills

2. PPE should include gloves, gowns, and facial protection.
3. Wipe up contaminated material or spilled material with disinfectant-soaked paper towels.
4. Rinse materials with water if necessary.
5. Discard all materials in a biohazardous-waste container.
6. Wash hands with soap and water.

C. Cleanup of spills in biological safety cabinet (BSC)

1. See procedure for operation of the BSC within this document
2. *Do not* turn off the cabinet.
3. Pour appropriate disinfectant for the agent over the spill area and then apply paper towels to absorb liquids. *Do not* use alcohol.
4. Allow 20 min of contact with the disinfectant.
5. Discard paper towels in a biohazardous-waste container.
6. Using a soft cloth soaked in disinfectant, wipe down all cabinet surfaces and equipment as needed.
7. If spills leak through the vent cover, remove and clean the gutter area with disinfectant.
8. Place gowns in a container for autoclaving after the cleanup process is completed.
9. Allow the cabinet blower to run for 10 min before resuming activity.
10. For major room spills of potentially infectious materials, contact a technical service consultant for decontamination.
11. *Do not* turn off the cabinet.

Limitations of the Procedure

A. *Do not* pour hypochlorite solutions into pools of urine, blood, or feces. Highly irritating gases may be produced.
B. *Do not* use low-level disinfectants, such as quaternary ammonium compounds, for disinfecting spills.

Quality Assurance

See the *Incident Response Plan* for reporting procedure.

L. AGENT SPECIFIC BIOSAFETY

Agent: Botulinum toxin

Pathogenicity:

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

Infectious Dose:

BoNT is one of the most toxic proteins known; absorption of less than one microgram (µg) or 0.001 µg/kg of BoNT (e.g. Type A) can cause severe incapacitation or death, depending upon the serotype and the route of exposure.

Host Range:

Four of the serotypes (A, B, E and, less commonly, F) are responsible for most human poisoning through contaminated food, wound infection, or infant botulism, whereas livestock may be at greater risk for poisoning with serotypes C1 and D.

Mode of Transmission:

Ingestion of contaminated food containing toxin. It is important to recognize, however, that all BoNT serotypes are highly toxic and lethal by injection or aerosol delivery.

Incubation Period:

6 hrs to 10 days after ingestion of toxin.

Communicability:

No person to person transmission.

Medical Surveillance:

Monitor for symptoms; demonstration of toxin in serum, stool, gastric aspirate or implicated food.

Immunizations / Vaccines

A pentavalent (A, B, C, D and E) botulinum toxoid vaccine (PBT) is available through the CDC as an IND. Vaccination is recommended for all personnel working in direct contact with cultures of *C. botulinum* or stock solutions of BoNT. Due to a possible decline in the immunogenicity of available PBT stocks for some toxin serotypes, the

immunization schedule for the PBT recently has been modified to require injections at 0, 2, 12, and 24 weeks, followed by a booster at 12 months and annual boosters thereafter.

Passive Immunization Experimental studies in NHP have demonstrated that administration of purified equine or human immune globulin may also confer passive protection against inhalation or injection of toxin.

Emergency Response to Potential Exposure

In case of Oral, Inhalation, Dermal, and/or Eye Exposure to Botulinum Toxin:

- ☐ Refer to this Material Safety Data Sheet (MSDS) for *Clostridium botulinum*
- ☐ If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately.
- ☐ If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.
- ☐ In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes.
- ☐ In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers.
- ☐ Notify Supervisor, Responsible Official, or Alternate Responsible Official. (In the absence of the supervisor contact first Joan Sturgeon for technical support)

Post-Exposure Treatment

Consultation with the local Health Officer or DHS-Division of Communicable Disease Control at (510) 540-2566; for DHS-Microbial Diseases Laboratory call (510) 412-3700 should be done ASAP.

See ***Diagnosis of Laboratory Exposures*** below for details.

Laboratory Hazards:

Laboratory Acquired Infections:

A documented incident of laboratory intoxication with BoNT occurred in workers who were performing necropsies on animals that had been exposed 24 h earlier to aerosolized BoNT serotype A; the laboratory workers presumably inhaled aerosols generated from the animal fur. The intoxications were relatively mild, and all affected

individuals recovered after a week of hospitalization. Despite the low incidence of laboratory-associated botulism, the remarkable toxicity of BoNT necessitates that laboratory workers exercise caution during all experimental procedures.

Sources/Specimens:

Person processing samples should never work alone.
Process one sample at a time under the BSC.
Open tube samples with an aerosol barrier, such as gauze or KimWipe.

After working with each sample, disinfect with 0.1 N NaOH.

Special Hazards:

Because *C. botulinum* requires an anaerobic environment for growth and it is essentially not transmissible among individuals, exposure to preformed BoNT is the primary concern for laboratory workers. Two of the most significant hazards in working with BoNT or growing *C. botulinum* cultures are unintentional aerosol generation, especially during centrifugation, and accidental needle-stick. Although BoNT does not penetrate intact skin, proteins can be absorbed through broken or lacerated skin and, therefore, BoNT samples or contaminated material should be handled with gloves.

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

Laboratory Facilities:

Containment Recommendations

BSL-2 practices, containment equipment, and facilities are recommended for routine dilutions, titrations or diagnostic studies with materials known to contain or have the potential to contain BoNT.

Additional primary containment and personnel precautions, such as those recommended for BSL-3, should be implemented for activities with a high potential for aerosol or droplet production, or for those requiring routine handling of larger quantities of toxin.

Non-immunized personnel should be discouraged from entering the laboratory when BoNT is in use until after the toxin and all work surfaces have been decontaminated. Purified preparations of toxin components, e.g. isolated BoNT “light chains” or “heavy chains,” should be handled as if contaminated with holotoxin unless proven otherwise by toxicity bioassays.

Personal Protective Equipment (PPE)

PPE for Laboratory Personnel:

BSL-2 or BSL-3 PPE depending on the activity *(see above under containment recommendations)*

Decontamination and Disposal

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.

Decontaminate surfaces and used non-disposable equipment with 0.1 N NaOH followed by 0.5% Sodium Hypochlorite (Bleach).

Autoclave all biohazard waste and used PPE at 121°C for 90 minutes immediately after processing specimens.

Wash hands with soap and water following the removal of PPE

Occupational Infections/Intoxication

Diagnosis of Laboratory Exposures

Botulism is primarily a clinical diagnosis with signs and symptoms that are similar for all serotypes and all routes of intoxication.There typically is a latency of several hours to days, depending upon the amount of toxin absorbed, before the signs and symptoms of BoNT poisoning occur. The first symptoms of exposure generally include blurred vision, dry mouth and difficulty swallowing and speaking. This is followed by a descending, symmetrical flaccid paralysis, which can progress to generalized muscle weakness and respiratory failure. Sophisticated tests such as nerve conduction studies and single-fiber electromyography can support the diagnosis and distinguish it from similar neuromuscular conditions.

Routine laboratory tests are of limited value because of the low levels of BoNT required to intoxicate, as well as the delay in onset of symptoms. Depending upon exposure levels, immunoassays may detect toxin in stool samples, gastric secretions, or respiratory secretions. BoNT is usually below detection in the serum or stool, but generally will be detectable on the nasal mucous membranes by immunoassay for 24 hours (h) after inhalation. Antibody levels in exposed individuals are not useful for diagnosing botulism, as a supralethal dose of BoNT may be too low to stimulate a measurable antibody titer.

Training

Training specific to Botulinum toxin is required and must be documented for all laboratory personnel working with Botulinum toxin, before starting work with the toxin and at intervals thereafter.

Agent: Staphylococcal enterotoxins

Physical Characteristics

Staphylococcal enterotoxins (SE) are a group of closely related extracellular protein toxins of 23 to 29 kD molecular weight that are produced by distinct gene clusters found in a wide variety of *S. aureus* strains.

Health Hazard Information

Pathogenicity:

SE belong to a large family of homologous pyrogenic exotoxins from staphylococci, streptococci and mycoplasma which are capable of causing a range of illnesses in man through pathological amplification of the normal T- cell receptor response, cytokine/ lymphokine release, immunosuppression and endotoxic shock. SE serotype A (SEA) is a common cause of severe gastroenteritis in humans.

Infectious Dose:

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

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

Mode of Transmission:

SE are highly toxic by intravenous and inhalation routes of exposure. Exposure of mucous membranes to SE in a laboratory setting has been reported to cause incapacitating gastrointestinal symptoms, conjunctivitis and localized cutaneous swelling.

Incubation Period:

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

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

Communicability:

None

Immunizations / Vaccines

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

Medical Surveillance / Post-Exposure Treatment

Diagnosis of Laboratory Exposures

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

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

Laboratory Facilities:

Containment Recommendations

BSL-2 practices and containment equipment and facilities should be used when handling SE or potentially contaminated material. Because SE is highly active by the oral or ocular exposure route, the use of a laboratory coat, gloves and safety glasses is mandatory when handling toxin or toxin-contaminated solutions. Frequent and careful hand-washing and laboratory decontamination should be strictly enforced when working

with SE. Depending upon a risk assessment of the laboratory operation, the use of a disposable face mask may be required to avoid accidental ingestion.

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

Laboratory Hazards:

Sources/Specimens:

Clinical samples, lesion fluids, respiratory secretions, or tissues of exposed animals.

Primary Hazards:

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

Special Hazards:

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

Personal Protective Equipment (PPE)

PPE for Laboratory Personnel:

BSL-2 or BSL-3 PPE depending on the activity *(see above under containment recommendations)*

PPE for Emergency Responders, Cleanup Personnel, and Investigators

BSL-2 or BSL-3 PPE depending on the activity *(see above under containment recommendations)*

Decontamination and Disposal

Occupational Intoxication

Accidental laboratory exposures to SE serotype B have been reviewed. Documented accidents included inhalation of SE aerosols generated from pressurized equipment failure, as well as re-aerosolization of residual toxin from the fur of exposed animals. The most common cause of laboratory intoxication with SE is expected to result from accidental self-exposure via the mucous membranes by touching contaminated hands to the face or eyes.

Training

Emergency Response to Potential Exposure

Wash hands with soap and water; shower with soap and water; prompt treatment with prophylaxis.

Decontamination and Disposal

Decontamination is defined as the irreversible inactivation of infectious agents so that an area is rendered safe. However, decontamination may not eliminate bacterial spores. Sterilization is the complete destruction or elimination of microbial viability, including spores.

The U.S. Environmental Protection Agency indicates that use of sodium hypochlorite as a sporicide is applicable under an emergency exemption (Section 18: Crisis Exemption; Federal Insecticide, Fungicide, and Rodenticide Act); as such, sodium hypochlorite may be used under the conditions specified.

- 10% acidified household bleach (0.5% sodium hypochlorite). Exposure for 30-60 minutes is effective.

Agent: Bacillus anthracis

Physical Characteristics

Aerobic, large gram positive rods occurring in chains; non-motile; forms resistant spores.

In addition to clinical specimens, *Bacillus anthracis* can found in

- A liquid (broth) form
- Culture form
- Dry powder spore form
- Lyophilized form

Attenuated Strains of *B. anthracis* excluded

Bacillus anthracis strains that are devoid of both virulence plasmids, pX01 and pX02 are excluded based on published studies evaluating the attenuation of strains containing different combinations of the two plasmids.

Bacillus anthracis strains lacking the virulence plasmid pX02 (e.g., Sterne $pX01^{+}$ and $pX02^{-}$) are excluded based on information indicating that these strains were 10^{5} to 10^{7} fold less virulent than isogenic strains with both plasmids. These strains have been used to vaccinate both humans and animals and do not pose a severe threat to the public health and safety.

Health Hazard Information

Direct and indirect contact of the intact and broken skin with cultures and contaminated laboratory surfaces; accidental parenteral inoculation and rarely, exposure to infectious aerosols are the primary hazards to laboratory personnel.

Pathogenicity:

B. anthracis exotoxin(s) consists of 3 components: the *edema factor* and *lethal factor* exert their effect within cells by interacting with a common transport protein, designated *protective antigen* (so named because, when modified, it contributes to vaccine efficacy). Expression of toxic factors is mediated by one plasmid and that of the capsule (D-glutamic acid polypeptide) is mediated by a second plasmid. Strains repeatedly subcultured at 42°C become avirulent as a result of losing virulence-determining plasmids; this loss is thought to be the basis for Pasteur's attenuated anthrax vaccine used at Pouilly-le-Fort, France, in 1881.

Cutaneous anthrax – itching, boils, and formation of a black scab (5-20% case fatality in untreated cases)
Inhalation anthrax – fever, chest pain, & difficulty breathing (usually fatal).
Gastrointestinal anthrax – abdominal distress, fever, nausea, vomiting, & diarrhea (25-60% case fatality)

Infectious Dose:

8,000 to 50,000 spores by inhalation

Host Range

Humans, cattle, sheep, goats, horses, pigs

Mode of Transmission:

Infection of skin by contact; inhalation anthrax results from inhalation of spores; gastrointestinal anthrax results from ingestion of spores.

Incubation Period:

Cutaneous anthrax: 1-12 days
Gastrointestinal anthrax: 1-7 days
Inhalational anthrax (human): 1-7 days, and may be as much as 60 days.

Communicability:

None.

Laboratory Safety

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.

Laboratory Facilities

Containment:

BSL-2 practices, containment equipment, and facilities are recommended for activities using ***clinical materials*** and diagnostic quantities of infectious ***cultures***.

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

Medical Surveillance:

Monitor for suspicious skin lesions and other symptoms; laboratory confirmation through direct microscopy, culture, immunological techniques.

Vaccines

A licensed vaccine for anthrax is available. Guidelines for its use in occupational settings are available from the ACIP. 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

Post Exposure Prophylaxis (PEP)

Antibiotic treatment (oral ciprofloxacin or doxycycline)

Emergency Response to Potential Exposure

Wash hands with soap and water; shower with soap and water; prompt treatment with prophylaxis.

Laboratory Hazards:

Laboratory Acquired Infections:

45 cases with 5 deaths occurring primarily in facilities conducting anthrax research; 25 reported cases of cutaneous anthrax among armed forces personnel

Sources/Specimens:

Blood, skin lesion exudates, and rarely in urine and feces; hides, hair, wool, bone and bone products, and tissues from infected animals**.** Persists in soil for ~40 years.

Primary Hazards:

Direct and indirect contact of skin with cultures and contaminated laboratory surfaces; accidental parenteral inoculation; exposure to infectious aerosols

Special Hazards:

- Naturally and experimentally infected animals pose a risk to laboratory and animal care personnel.
- Preparing slides, vortexing tubes, or for activities with a high potential for aerosol production, must be placed inside a certified biological safety cabinet (BSC) to minimize exposure to potential aerosols.
- When handling suspected dry forms of *Bacillus anthracis* two individuals must be present in the room and they must wear respirators equipped with HEPA filters.

Personal Protective Equipment (PPE)

PPE for Laboratory Personnel:

When handling *Bacillus anthracis* directly or in a manner in which exposure might occur, the following procedures apply:

1. Wear nitrile gloves
2. Wear eye protection
    a. When working with liquid forms of the *Bacillus anthracis:*
        1) Faceshields are to be worn when using a disposable N-95 or P-100 filter respirator.
    b. When working with dry forms of the *Bacillus anthracis*:
        1) A PAPR that provides eye protection must be worn
3. Respiratory Protection
    a. When working with dry forms of the *Bacillus anthracis*:
        1) A PAPR that provides eye protection must be worn

PPE for Emergency Responders, Cleanup Personnel, and Investigators

See the “Red Zone” PPE guidance in OSHA’s Anthrax Matrix (http://www.osha.gov/dep/anthrax/anthraxmatrixred.html)

Decontamination and Disposal

Decontamination is defined as the irreversible inactivation of infectious agents so that an area is rendered safe. However, decontamination may not eliminate bacterial spores. Sterilization is the complete destruction or elimination of microbial viability, including spores.

The U.S. Environmental Protection Agency indicates that use of sodium hypochlorite as a sporicide is applicable under an emergency exemption (Section 18: Crisis Exemption; Federal Insecticide, Fungicide, and Rodenticide Act); as such, sodium hypochlorite may be used under the conditions specified.

- 10% acidified household bleach (0.5% sodium hypochlorite). Exposure for 30-60 minutes is effective. Organic matter may decrease the sporicidal efficiency of sodium hypochlorite.

Table 1. Heat inactivation of *Bacillus anthracis* spores

Temperature	Time	Inoculum size	Inactivation effect
Boiling			
100°C	10 min	3×10^6	Sample sterilized
	5 min	7.5×10^8	Sample sterilized
Moist heat			
90°C	20 min	1.2×10^6	Sample sterilized
90°C to 91°C	60 min	3×10^8	Spores detected
100°C	10 min	1.2×10^6	Sample sterilized
100°C to 101°C	17 min	1×10^5	Sample sterilized
105°C	10 min	3×10^6	Sample sterilized
120°C	15 min	2.4×10^8	Sample sterilized
Dry heat			
140°C	>90 min	6×10^3 to 1.2×10^4	Sample sterilized
150°C	10 min	6×10^3 to 1.2×10^4	Sample sterilized
160°C	10 min	6×10^3 to 1.2×10^4	Sample sterilized
180°C	2 min	6×10^3 to 1.2×10^4	Sample sterilized
190°C	1 min	6×10^3 to 1.2×10^4	Sample sterilized
200°C	30 sec	6×10^3 to 1.2×10^4	Sample sterilized

[a]Spores in liquid suspension exposed to flowing steam at 100°C.

Training

Training specific to *Bacillus anthracis* is required and must be documented for all laboratory personnel working with *Bacillus anthracis*, before starting work with the agent and at intervals thereafter.

Agent: Brucella species

Physical Characteristics

Gram negative cocci or small rods, aerobic, non-motile, urease +

In addition to clinical specimens, *Brucella (B. abortus, B. canis, B. melitensis, B. suis)* can found in

1. A liquid (broth) form
2. Culture form
3. Lyophilized form

Attenuated strains of *Brucella (B. abortus, B. canis, B. melitensis, B. suis*) excluded:

The *Brucella abortus* Strain 19 live vaccine, used in the U.S. Department of Agriculture Brucellosis Eradication Program from 1941 to 1996, is effective in the control of clinical brucellosis in cattle.[16] For over a decade, *B. abortus* Strain 19 was also used to immunize more than 8 million people in the USSR.[17] While there have been occasional reports of human brucellosis caused by *B. abortus* Strain 19 as a result of accidental aerosolization or needle sticks, [18, 19] this strain does not pose a severe threat to human or animal health.

Brucella abortus strain RB51 was conditionally licensed as a vaccine by USDA in 1996 and granted a full license in March 2003. It is used as part of the cooperative State-Federal Brucellosis Eradication Program.[16] Brucella abortus strain RB51 is a genetically stable, rough morphology mutant of field strain Brucella. It lacks the polysaccharide O-side chains on the surface of the bacteria. Strain RB51 is less virulent than the Brucella abortus Strain 19 vaccine and field strain[17] Brucella abortus. The RB51 strain does not pose a significant threat to human or animal health.

Health Hazard Information

Direct and indirect contact of the intact and broken skin with cultures and contaminated laboratory surfaces; accidental parenteral inoculation and rarely, exposure to infectious aerosols are the primary hazards to laboratory personnel.

Occupational Infections

Brucellosis has been one of the most frequently reported laboratory infections in the past and cases continue to occur. Airborne and mucocutaneous exposures can produce LAI.

Pathogenicity:

All *Brucella* isolates are potentially pathogenic to humans; systemic bacterial disease with acute or insidious onset; intermittent fever, headache, weakness, profuse sweating, chills, arthralgia; localized suppurative infections; subclinical infections are frequent; <2% case fatality rate for untreated cases; may have long recovery period.

The major virulence factor for brucellosis appears to be an endotoxic lipopolysaccharide (LPS) among smooth strains. Pathogenicity is related to an LPS containing poly *N*-formyl perosamine O chain, Cu-Zn, superoxide dismutase, erythrulose phosphate dehydrogenase, intracellular survival stress-induced proteins, and adenine- and guanine-monophosphate inhibitors of phagocyte functions. PMN, polymorphonuclear leukocyte.

Infectious Dose:

10-100 organisms.

Host Range

Humans, cattle, swine, goats, sheep, deer, caribou, elk, dogs, coyotes

Mode of Transmission:

Through ingestion, direct contact via skin abrasions and mucous membranes, and inhalation; risk factors include contact with infected tissues, blood, urine, vaginal discharge, aborted fetuses; ingestion of raw milk or cheese from infected animals; contact in abattoirs; laboratory-acquired (generally through aerosolization)

Incubation Period:

Highly variable; 5- 60 days; occasionally several months

Communicability:

No evidence of person-to-person transmission.

Medical Surveillance:

Monitor for symptoms; isolation of organism from blood or tissue samples; confirm by serological testing

Immunizations / 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.

Post Exposure Prophylaxis (PEP):

Generally the antibiotics doxycycline and rifampin are recommended in combination for a minimum of 6-8 weeks. Let your doctor know if you are:

- pregnant
- allergic to doxycycline or rifampin
- suffer from a reduced or absent immune response (immunosupressed)

Emergency Response to Potential Exposure

Wash hands with soap and water; shower with soap and water.

Laboratory Facilities

Containment Recommendations

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.

Laboratory Hazards:

Laboratory Acquired Infections:

Brucellosis continues to be the most commonly reported laboratory-associated bacterial infection. *B. abortus, B. canis, B. melitensis*, and *B. suis* have all caused illness in laboratory personnel. Hypersensitivity to *Brucella* antigens is also a hazard to laboratory personnel. Occasional cases have been attributed to exposure to experimentally and naturally infected animals or their tissues.

The agent may be present in blood, cerebrospinal fluid, semen, and occasionally urine. Most laboratory-associated cases have occurred in research facilities and have involved exposure to *Brucella* organisms grown in large quantities. Cases have also occurred in the clinical laboratory setting from sniffing bacteriological cultures. Direct skin contact with cultures or with infectious clinical specimens from animals (e.g., blood, uterine

discharges) are commonly implicated in these cases. Aerosols generated during laboratory procedures have caused large outbreaks. Mouth pipetting, accidental parenteral inoculations, and sprays into eyes, nose, and mouth have also resulted in infection. [6]

Laboratory Safety

Brucella infects the blood and a wide variety of body tissues, including cerebral spinal fluid, semen, pulmonary excretions, placenta, and occasionally urine. Most laboratory-associated cases occur in research facilities and involve exposures to *Brucella* organisms grown in large quantities or exposure to placental tissues containing *Brucella*. Cases have occurred in clinical laboratory settings from sniffing bacteriological

cultures or working on open bench tops. 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. 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.

Sources/Specimens:

Cultures, blood, tissues, placentas, fetuses, urine, uterine discharges. Persists in water or soil for ~10 weeks.

Primary Hazards:

Exposure to aerosols; direct skin contact with cultures of infectious specimens from animals; ingestion (mouth pipetting); accidental inoculation; sprays into eyes, nose and mouth.

Special Hazards:

- Naturally and experimentally infected animals pose a risk to laboratory and animal care personnel.
- Preparing slides, vortexing tubes, or for activities with a high potential for aerosol production, must be placed inside a certified biological safety cabinet (BSC) to minimize exposure to potential aerosols.
- When handling suspected dry forms of *Brucella (B. abortus, B. canis, B. melitensis, B. suis)* two individuals must be present in the room and they must wear respirators equipped with HEPA filters.

Personal Protective Equipment (PPE)

PPE for Laboratory Personnel:

When handling *Brucella (B. abortus, B. canis, B. melitensis, B. suis)* directly or in a manner in which exposure might occur, the following procedures apply:

1. Wear nitrile gloves
2. Wear eye protection
    a. When working with liquid forms of the *Brucella (B. abortus, B. canis, B. melitensis, B. suis)*
        1) A face shield is are to be worn when using a disposable N-95 or P100 filter mask.
    b. When working with dry forms of the *Brucella (B. abortus, B. canis, B. melitensis, B. suis)*
        1) A PAPR that provides eye protection must be worn
3. Respiratory Protection (See "Guideline for Work With Select Agents and Toxins")
    a. When working with dry forms of the *Brucella (B. abortus, B. canis, B. melitensis, B. suis)*
        1) A PAPR that provides eye protection must be worn

PPE for Emergency Responders, Cleanup Personnel, and Investigators

See the "Red Zone" PPE guidance in OSHA's Anthrax Matrix (http://www.osha.gov/dep/anthrax/anthraxmatrixred.html)

Decontamination and Disposal

Decontamination is defined as the irreversible inactivation of infectious agents so that an area is rendered safe. However, decontamination may not eliminate bacterial spores. Sterilization is the complete destruction or elimination of microbial viability, including spores.

The U.S. Environmental Protection Agency indicates that use of sodium hypochlorite as a sporicide is applicable under an emergency exemption (Section 18: Crisis Exemption; Federal Insecticide, Fungicide, and Rodenticide Act); as such, sodium hypochlorite may be used under the conditions specified.

- 10% acidified household bleach (0.5% sodium hypochlorite). Exposure for 30-60 minutes is effective.

Training

Training specific to *Brucella (B. abortus, B. canis, B. melitensis, B. suis)* is required and must be documented for all laboratory personnel working with *Brucella (B. abortus, B. canis, B. melitensis, B. suis)*, before starting work with the agent and at intervals thereafter.

Agent: Burkholderia mallei

Physical Characteristics

Gram-negative rods, aerobic, nonmotile, bipolar staining, nonpigmented.

In addition to clinical specimens *Burkholderia mallei* can found in

1. A liquid (broth) form
2. Culture form
3. Lyophilized form

Attenuated strains of Burkholderia mallei excluded:

None, currently.

Health Hazard Information

Glanders is a bacterial infection caused by the Gram-negative rod, B. mallei (formerly Pseudomonas mallei). Primarily a disease of equids (e.g., horses, mules, and donkeys), glanders also has been reported in carnivores that have fed on infected horse carcasses and, although rare, glanders has been reported in humans. The disease was eliminated from domestic animals in the United States during the 1940s and the last reported human case in the United States occurred in 1945. Glanders still occurs occasionally in equids and humans in central and southeast Asia, the Middle East, parts of Africa, and possibly South America, and B. mallei is being researched in the United States because it is considered a potential agent of biological terrorism.

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

Pathogenicity:

In humans, glanders usually is acquired through direct skin or mucous membrane contact with infected animal tissues. The incubation period usually is 1 to 14 days. The clinical presentation varies; cutaneous inoculation can result in localized infection with nodule formation and lymphadenitis. The disease often manifests as pneumonia, bronchopneumonia, or lobar pneumonia, with or without bacteremia. Hepatic and splenic involvement has been reported. [15]

Infectious Dose:

Unknown.

Host Range:
Equines, especially horses and mules; humans are accidental hosts

Mode of Transmission:
Direct contact with nasal secretion of equines; inhalation of aerosols.

Incubation Period:
1-14 days

Communicability:
Handling cultures shown to be high risk for infection.

Medical Surveillance:
Monitor for symptoms and isolate patients; use mallein in intrapalpebral test to detect infected equines for slaughter; isolate and characterize organism to confirm.

Immunization:
No vaccine against B. mallei infection is available.

Post Exposure Prophylaxis (PEP):
A few antibiotics have been used to treat humans. Sulfadiazine (25 mg/kg intravenous, four times a day) was efficacious in some cases. In mice, doxycycline and ciprofloxacin have been effective therapies. The mortality of apparent infection was approximately 95% before the use of antimicrobial agents; however, except when bacteremia develops, better diagnosis and more appropriate therapy have lowered mortality. [15]

Emergency Response to Potential Exposure

Wash hands with soap and water; shower with soap and water.

Laboratory Facilities

Containment Recommendations
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.

Laboratory Safety

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. Laboratory-acquired infections have resulted from aerosol and cutaneous exposure. 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.

Laboratory Hazards:

Laboratory Acquired Infections:

Glanders has been reported as a laboratory-acquired infection. During World War II, six unrelated cases of laboratory-acquired infection with B. mallei occurred at Camp Detrick, Frederick, Maryland. Some of these cases were attributed to inhalation of infectious aerosols generated by spillages of liquid culture media containing the bacterium. Other cases were reported to have no obvious cause other than the routine handling of the organism. The presentation of unilateral lymphadenopathy suggests a cutaneous inoculation. Most laboratory-acquired infections are associated with routine handling of microbes and not with injuries

Sources/Specimens:

Sputum, blood, and wound exudate.

Primary Hazards:

Aerosol and cutaneous inoculation.

Special Hazards:

- Naturally and experimentally infected animals pose a risk to laboratory and animal care personnel.
- Preparing slides, vortexing tubes, or for activities with a high potential for aerosol production, must be placed inside a certified biological safety cabinet (BSC) to minimize exposure to potential aerosols.
- When handling suspected dry forms *Burkholderia mallei* two individuals must be present in the room and they must wear respirators equipped with HEPA filters.

Personal Protective Equipment (PPE)

PPE for Laboratory Personnel:

When handling *Burkholderia mallei* directly or in a manner in which exposure might occur, the following procedures apply:

1. Wear nitrile gloves
2. Wear eye protection
    a. When working with liquid forms of the *Burkholderia mallei* A face shield is to be worn when using a disposable N-95 or P100 filter mask.
    b. When working with dry forms of the *Burkholderia mallei)*
        1) A PAPR that provides eye protection must be worn
3. Respiratory Protection
    a. When working with dry forms of the *Burkholderia mallei*
        1) A PAPR that provides eye protection must be worn

PPE for Emergency Responders, Cleanup Personnel, and Investigators

See the "Red Zone" PPE guidance in OSHA's Anthrax Matrix (http://www.osha.gov/dep/anthrax/anthraxmatrixred.html)

Decontamination and Disposal

Decontamination is defined as the irreversible inactivation of infectious agents so that an area is rendered safe. However, decontamination may not eliminate bacterial spores. Sterilization is the complete destruction or elimination of microbial viability, including spores.

The U.S. Environmental Protection Agency indicates that use of sodium hypochlorite as a sporicide is applicable under an emergency exemption (Section 18: Crisis Exemption; Federal Insecticide, Fungicide, and Rodenticide Act); as such, sodium hypochlorite may be used under the conditions specified.

- 10% acidified household bleach (0.5% sodium hypochlorite). Exposure for 30-60 minutes is effective.

Storage

LACPHL is a non-Tier 1 laboratory and as such, must destroy or transfer the agent within 7 days of identification.

Training

Training specific to *Burkholderia mallei* is required and must be documented for all laboratory personnel working *Burkholderia mallei* before starting work with the agent and at intervals thereafter.

Agent: Burkholderia pseudomallei

Physical Characteristics

Gram-negative rod, motile, aerobic, young cultures exhibit bipolar staining (safety pin appearance), wrinkled colonies on agar media.

In addition to clinical specimens *Burkholderia pseudomallei* can found in

1. A liquid (broth) form
2. Culture form
3. Lyophilized form

Attenuated strains of *Burkholderia pseudomallei* excluded:

None, currently.

Health Hazard Information

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. Laboratory workers with diabetes are at increased risk of contracting melioidosis.

Pathogenicity:

Melioidosis - an endemic glanders-like disease; clinical symptoms vary from inapparent infection to chronic infection to rapidly fatal septicemia; may simulate typhoid fever or more commonly tuberculosis, with symptoms such as empyema, chronic abscesses and osteomyelitis.

Infectious Dose:

Unknown.

Host Range

Humans and animals. Environmental organism found in certain waters and soils; animals include sheep, goats, horses, swine, monkey and rodents

Mode of Transmission:

Acquired by ingestion, inhalation or contact of abraded, wounded or burned skin with contaminated water or soil.

Incubation Period:

2 days (several months or years may elapse between presumed exposure and clinical disease)

Communicability:

Person to person transmission is extremely rare; human carriers are not known.

Medical Surveillance:

Monitor for symptoms; confirm by rise in antibody titre and isolation of organism.

Immunization:

None.

Post Exposure Prophylaxis (PEP):

None.

Emergency Response to Potential Exposure

Wash hands with soap and water; shower with soap and water.

Laboratory Facilities

Containment Recommendations

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.

Laboratory Safety

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.

Laboratory Hazards:

Laboratory Acquired Infections:

Two laboratory-associated cases of melioidosis have been reported: one associated with a massive aerosol and skin exposure; the second resulting from an aerosol created during the open-flask sonication of a culture presumed to be *P. cepacia*.

Sources/Specimens:

Sputa, blood, wound exudates, tissues.

Primary Hazards:

The agent may be present in sputum, blood, wound exudates and various tissues depending on the infection's site of localization. Direct contact with cultures and infectious materials from humans, animals, or the environment, ingestion, auto-inoculation, and exposure to infectious aerosols and droplets are the primary laboratory hazards. The agent has been demonstrated in blood, sputum, and abscess materials and may be present in soil and water samples from endemic areas.

Special Hazards:

- Naturally and experimentally infected animals pose a risk to laboratory and animal care personnel.
- Preparing slides, vortexing tubes, or for activities with a high potential for aerosol production, must be placed inside a certified biological safety cabinet (BSC) to minimize exposure to potential aerosols.
- When handling suspected dry forms *Burkholderia pseudomallei* two individuals must be present in the room and they must wear respirators equipped with HEPA filters.

Personal Protective Equipment (PPE)

PPE for Laboratory Personnel:

When handling *Burkholderia pseudomallei* directly or in a manner in which exposure might occur, the following procedures apply:

1. Wear nitrile gloves
2. Wear eye protection
    a. When working with liquid forms of the *Burkholderia pseudomallei* a faceshield
    b. is to be worn when using a disposable N-95 or P100 filter mask.
    c. When working with dry forms of the *Burkholderia pseudomallei)*
        1) A PAPR that provides eye protection must be worn
3. Respiratory Protection
    a. When working with dry forms of the *Burkholderia pseudomallei*
        1) A PAPR that provides eye protection must be worn

PPE for Emergency Responders, Cleanup Personnel, and Investigators

See the “Red Zone” PPE guidance in OSHA’s Anthrax Matrix
http://www.osha.gov/dep/anthrax/anthraxmatrixred.html

Decontamination and Disposal

Decontamination is defined as the irreversible inactivation of infectious agents so that an area is rendered safe. However, decontamination may not eliminate bacterial spores. Sterilization is the complete destruction or elimination of microbial viability, including spores.

The U.S. Environmental Protection Agency indicates that use of sodium hypochlorite as a sporicide is applicable under an emergency exemption (Section 18: Crisis Exemption; Federal Insecticide, Fungicide, and Rodenticide Act); as such, sodium hypochlorite may be used under the conditions specified.

- 10% acidified household bleach (0.5% sodium hypochlorite). Exposure for 30-60 minutes is effective.

Storage

LACPHL is a non-Tier 1 laboratory and as such, must destroy or transfer the agent within 7 days of identification.

Training

Training specific to *Burkholderia pseudomallei* is required and must be documented for all laboratory personnel working *Burkholderia pseudomallei* before starting work with the agent and at intervals thereafter.

Agent: Botulinum producing species of *Clostridium*

Physical Characteristics

Gram positive rods, spore-former, anaerobic, produces neurotoxin under anaerobic conditions and especially in low-acid foods

In addition to clinical specimens *Botulinum producing species of Clostridium* can found in

- A liquid (broth) form
- Culture form
- Lyophilized form

Amounts Permissible:

Tier 1 Agent select agent laboratories only.

Health Hazard Information

Pathogenicity:

Three forms of botulism, all caused by the neurotoxin which binds irreversibly at the neuromuscular junctions of motor neurons:
(1) Foodborne: rare, potentially life-threatening; caused by the ingestion of preformed botulinum toxin in contaminated food; characterized by acute flaccid paralysis involving the muscles of the face, head and pharynx, down to the thorax and extremities; death may result from respiratory failure;
(2) Wound botulism: occurs subsequent to the growth of the organism in a contaminated wound; toxin is released into the bloodstream; same symptoms as above;
(3) Infant botulism: results from spore ingestion and subsequent growth and toxin production in the intestinal tract; affects infants under 1 year almost exclusively; wide spectrum of clinical severity

Infectious Dose:

Unknown for infant botulism: cells/spores not normally toxic to adults; toxin is extremely potent.

Host Range:

Humans, animals including fish

Mode of Transmission:

Ingestion of contaminated food containing toxin.

Incubation Period:

12 - 36 hrs after ingestion of toxin.

Communicability:

No person to person transmission.

Medical Surveillance:

Monitor for symptoms; demonstration of toxin in serum, stool, gastric aspirate or implicated food.

Immunizations / Vaccines

A pentavalent (A, B, C, D and E) botulinum toxoid vaccine (PBT) is available through the CDC as an IND. Vaccination is recommended for all personnel working in direct contact with cultures of *C. botulinum* or stock solutions of botulinum neurotoxin (BoNT). Due to a recent decline in 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.

Emergency Response to Potential Exposure

Wash hands with soap and water; shower with soap and water.

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.

Laboratory Facilities:

Containment:

BSL-2 practices, containment equipment, and facilities are recommended for activities that involve the organism or the toxin 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.

BSL-3 practices, containment equipment, and facilities are required for activities with a high potential for aerosol or droplet production, and for those involving large quantities of the organism or of the toxin. ABSL-2 practices, containment equipment, and facilities are recommended for diagnostic studies and titration of toxin.

Laboratory Hazards:

Laboratory Acquired Infections:

While there is only one report of botulism associated with the handling of the agent or toxin in the laboratory or working with naturally or experimentally infected animals, the consequences of such intoxications must still be considered quite grave. Work with cultures of *C. botulinum* requires special security considerations due to their potential use for purposes of biological terrorism.

Sources/Specimens:

Food products, clinical materials (serum, feces) and environmental samples (soil, surface water)

Primary Hazards:

Exposure to the toxin; absorbed after ingestion, or following contact with the skin, eyes, or mucous membranes including the respiratory tract; accidental parenteral inoculation.

C. botulinum or its toxin may be present in a variety of food products, clinical materials (serum, feces), and environmental samples (soil, surface water). Exposure to the toxin of *C. botulinum* is the primary laboratory hazard. The toxin may be absorbed after ingestion or following contact with the skin, eyes, or mucous membranes, including the respiratory tract. Accidental parenteral inoculation may also represent a significant exposure to toxin. Broth cultures grown under conditions of optimal toxin production may contain $2x10^6$ mouse LD_{50} per mL.

Special Hazards:

- Naturally and experimentally infected animals pose a risk to laboratory and animal care personnel.
- Preparing slides, vortexing tubes, or for activities with a high potential for aerosol production, must be placed inside a certified biological safety cabinet (BSC) to minimize exposure to potential aerosols.
- When handling suspected dry forms *Botulinum producing species of Clostridium* two individuals must be present in the room and they must wear respirators equipped with HEPA filters.

Personal Protective Equipment (PPE)

PPE for Laboratory Personnel:

When handling *Botulinum producing species of Clostridium* directly or in a manner in which exposure might occur, the following procedures apply:

1. Wear nitrile gloves
2. Wear eye protection
    a. When working with liquid forms of the *Botulinum producing species of Clostridium* a faceshield is to be worn when using a disposable N-95 or P-100 filter mask.

b. When working with dry forms of the *Botulinum producing species of Clostridium)*
    1) A PAPR that provides eye protection must be worn

3. Respiratory Protection
    a. When working with dry forms of the *Botulinum producing species of Clostridium*
        1) A PAPR that provides eye protection must be worn

PPE for Emergency Responders, Cleanup Personnel, and Investigators

See the "Red Zone" PPE guidance in OSHA's Anthrax Matrix (http://www.osha.gov/dep/anthrax/anthraxmatrixred.html)

Decontamination and Disposal

Decontamination is defined as the irreversible inactivation of infectious agents so that an area is rendered safe. However, decontamination may not eliminate bacterial spores. Sterilization is the complete destruction or elimination of microbial viability, including spores.

The U.S. Environmental Protection Agency indicates that use of sodium hypochlorite as a sporicide is applicable under an emergency exemption (Section 18: Crisis Exemption; Federal Insecticide, Fungicide, and Rodenticide Act); as such, sodium hypochlorite may be used under the conditions specified.

- 10% acidified household bleach (0.5% sodium hypochlorite) Exposure for 30-60 minutes is effective. Organic matter may decrease the sporicidal efficiency of sodium hypochlorite.

Storage

LACPHL is a non-Tier 1 laboratory and as such, must destroy or transfer the agent within 7 days of identification.

Occupational Intoxication

There has been only one report of botulism associated with handling of the toxin in a laboratory setting.

Laboratory Safety

C. botulinum or its toxin may be present in a variety of food products, clinical materials (serum, feces) and environmental samples (soil, surface water). In addition, bacterial cultures may produce very high levels of toxin. In healthy adults, it is typically the toxin and not the organism that causes disease.

Training

Training specific to *Botulinum producing species of Clostridium* is required and must be documented for all laboratory personnel working *Botulinum producing species of Clostridium* before starting work with the agent and at intervals thereafter.

Agent: Francisella tularensis

Physical Characteristics

Gram negative non motile coccobacillus, non-sporing, aerobic, requires cystine for growth, grows well on Legionella media (BCYE) and slowly on enriched (Columbia base) blood agar; two biovars, Jellison type A (highly virulent) and Jellison type b (mild disease)

In addition to clinical specimens *Francisella tularensis* can found in

1. A liquid (broth) form
2. Culture form
3. Lyophilized form

Attenuated strains of Francisella tularensis excluded:

Francisella tularensis subspecies _novicida_ (aka to as _Francisella novicida_) strain, Utah 112 (ATCC 15482). (effective 2-27-2003)

The type strain Utah 112 of *Francisella tularensis* subspecies *novicida* (also referred to as *Francisella novicida*) is excluded. The exclusion is only for the type strain, Utah 112. This strain was originally isolated from a water sample taken from Ogden Bay, Utah in 1951. It is experimentally pathogenic for mice, guinea pigs and hamsters, producing lesions similar to those of tularemia; rabbits, white rats and pigeons are resistant. The Utah 112 strain is not known to infect man and thus, is not of public health concern.

Francisella tularensis subspecies _holartica_ LVS (live vaccine strain; includes NDBR 101 lots, TSI-GSD lots, and ATCC 29684). (effective 2-27-2003)

Francisella tularensis subspecies *holartica* LVS (live vaccine strain) is excluded. This and similar strains have been used to vaccinate millions of people including thousands of U.S. military personnel and laboratory workers without major problems.

Francisella tularensis ATCC 6223 (aka strain B38). (effective 4-14-2003)

Francisella tularensis biovar *tularensis* strain ATCC 6223. This strain has fastidious growth requirements and grows poorly in the laboratory. Mice are used as a model to study the pathogenesis of tularemia. The LD50 of virulent strains of *F. tularensis* biovar *tularensis* for mice infected via the subcutaneous route is <10 CFU. However, mice infected intraperitoneally with 10^5 CFU or intradermally with 10^7 CFU of strain ATCC 6223 were not killed. Thus, strain ATCC 6223 does not pose a threat to human or animal health.

Health Hazard Information

Pathogenicity:

Human tularemia presents as an indolent ulcer at site of infection, accompanied by swelling of the regional lymph nodes (ulceroglandular); sudden on set of pain and fever, fever generally lasts 3 - 6 weeks without treatment; inhalation may be followed by a pneumonic disease or primary systemic (typhoidal) picture; type B strains 5-15% fatality rate; type A strains approximately 35% mortality from pulmonary tularemia.

Infectious Dose:

5 - 10 organisms by the respiratory route; 10^6 - 10^8 organisms by ingestion

Host Range:

Wild animals (rabbits) and birds; some domestic animals; humans

Mode of Transmission:

Inoculation of skin, conjunctival sac or oropharyngeal mucosa with blood or tissue while handling infected animals, or by fluids from infected flies, ticks or other animals; bite of arthropods (deerfly, mosquito) and ticks; ingestion of contaminated food and drinking water; inhalation of contaminated dust; able to pass through unbroken skin; rarely through bites of animals

Incubation Period:

Related to virulence of infecting strain, size of inoculum (dose) and route of introduction; 1 - 14 days, usually 3- 5 days

Communicability:

Not directly transmitted from person-to-person; unless treated, infectious agent may be found in blood during first 2 weeks of disease and in lesions for a month; flies infective for 14 days, and ticks throughout lifetime (2 years)

Medical Surveillance:

Monitor for symptoms; confirm by serological testing

Immunization:

Live attenuated vaccines available from CDC for occupational risk groups

Post Exposure Prophylaxis (PEP):

Antibiotic therapy with streptomycin

Emergency Response to Potential Exposure

Wash hands with soap and water; shower with soap and water.

Laboratory Facilities:

Containment Recommendations

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.

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.

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.

Manipulation of reduced virulence strains at high concentrations should be conducted using BSL-3 practices.

Laboratory Hazards:

Laboratory Acquired Infections:

Tularemia has been a commonly reported laboratory-associated bacterial infection. Almost all cases occurred at facilities involved in tularemia research. Occasional cases have been related to work with naturally or experimentally infected animals or their ectoparasites. Although not reported, cases have occurred in clinical laboratories. Work with cultures of *F. tularensis* requires special security considerations due to their potential use for purposes of biological terrorism.

Laboratory Safety

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.

Special Hazards:

- Naturally and experimentally infected animals pose a risk to laboratory and animal care personnel.
- Preparing slides, vortexing tubes, or for activities with a high potential for aerosol production, must be placed inside a certified biological safety cabinet (BSC) to minimize exposure to potential aerosols.

- When handling suspected dry forms *Francisella tularensis* two individuals must be present in the room and they must wear respirators equipped with HEPA filters.

Personal Protective Equipment (PPE)

PPE for Laboratory Personnel:

When handling *Francisella tularensis* directly or in a manner in which exposure might occur, the following procedures apply:

1. Wear nitrile gloves
2. Wear eye protection
    a. When working with liquid forms of the *Francisella tularensis* a faceshield is to be worn when using a disposable N-95 or P100 filter mask.
    b. When working with dry forms of the *Francisella tularensis)*
        1) A PAPR that provides eye protection must be worn
3. Respiratory Protection
    a. When working with dry forms of the *Francisella tularensis*
        1) A PAPR that provides eye protection must be worn

PPE for Emergency Responders, Cleanup Personnel, and Investigators

See the “Red Zone” PPE guidance in OSHA’s Anthrax Matrix (http://www.osha.gov/dep/anthrax/anthraxmatrixred.html)

Decontamination and Disposal

Decontamination is defined as the irreversible inactivation of infectious agents so that an area is rendered safe. However, decontamination may not eliminate bacterial spores. Sterilization is the complete destruction or elimination of microbial viability, including spores.

The U.S. Environmental Protection Agency indicates that use of sodium hypochlorite as a sporicide is applicable under an emergency exemption (Section 18: Crisis Exemption; Federal Insecticide, Fungicide, and Rodenticide Act); as such, sodium hypochlorite may be used under the conditions specified.

- 10% acidified household bleach (0.5% sodium hypochlorite)

Storage

LACPHL is a non-Tier 1 laboratory and as such, must destroy or transfer the agent within 7 days of identification.

Training

Training specific to *Francisella tularensis* is required and must be documented for all laboratory personnel working *Francisella tularensis* before starting work with the agent and at intervals thereafter.

Agent: Variola virus

Physical Characteristics

A member of the family *Poxviridae* , subfamily *Chordopoxvirinae* , genus *Orthopoxvirus*. Virions are shaped like bricks on electron micrographs and measure approximately 300 x 250 x 200 nm. *Orthopoxviruses* have an outside envelope and a second membrane underneath. Instead of a capsid, poxviruses have a nucleosome which contains DNA, and is surrounded by its own membrane. They contain single, linear, double-stranded DNA molecules of 130 to 375 kb pairs and replicate in the cell cytoplasm. Variola virus is the most complex of the *orthopoxvirus*genus, having many different strains.

Health Hazard Information

Pathogenicity:

Smallpox is an acute, contagious disease with two main forms, variola major and variola minor, both of which cause similar lesions.

There are 4 types of variola major infection presentations.

***Ordinary variola major*:** The most common form, which accounts for more than 90% of cases and has a fatality rate of approximately 30% among unvaccinated individuals, and 3% for vaccinated individuals

The cause of death is usually bronchopneumonia, although in about 3% of cases, fatal haemorrhages occur. The prodromal phase consists of sudden onset of influenza-like symptoms, characterized by fever, malaise, headache, prostration, severe back pain, and sometimes abdominal pain and vomiting. Two to 3 days later, the temperature falls and the patient feels somewhat better, at which time a characteristic rash appears, first on the face (starting as small red spots) then on the tongue, mouth, nose, and hands. After a few days, the rash progresses to the trunk where fewer lesions occur. Lesions in the mucous membranes of the nose and mouth ulcerate quickly, releasing large amounts of virus into the mouth and throat. Lesions progress from macules to papules to vesicles to pustules, and at 8 to14 days, the pustules form scabs which leave depressed depigmented scars upon healing. All lesions in a given area progress through these stages together.

***The milder or “"modified"” variola major*:** Accounts for 2% of cases in unvaccinated persons and for 25% in previously vaccinated persons. Cases are rarely fatal with fewer, smaller, and more superficial lesions than those in patients with the ordinary type.

***Hemorrhagic variola major*:** A rare form of variola major which is always fatal and involves hemorrhages in the mucous membranes and the skin
***Flat variola major*:** Another rare form of variola major that is almost always fatal and is characterized by lesions that do not develop to the pustular stage, but remain soft and

flat. *Variola minor*: The other main form of smallpox (also known as alastrim), which is a milder illness with a fatality rate of less than 1%

Another type of smallpox, *variola sine eruptione* , occurs in previously vaccinated contacts or in infants with maternal antibodies. Affected persons are asymptomatic or have a brief rise in temperature, headache, and present influenza-like symptoms. The transmission of clinical smallpox has not been documented with *variola sine eruptione*. Overall, 65% to 80% of smallpox survivors have pockmarks, mostly on the face.

Epidemiology:

As a result of a successful worldwide vaccination program, smallpox was eradicated, with the last natural infection occurring in Somalia in 1977.

Host Range:

Humans. Monkeys are also susceptible to infection

Infectious Dose:

10 to 100 particles

Mode of Transmission:

Transmission occurs via respiratory droplets (primary route of transmission), or via fine-particle aerosol, or skin inoculation. The conjunctiva or placenta may be occasional portals of entry.

Respiratory droplets (i.e., coughing, sputum, and saliva) have a range of likely no more than 2 meters and are, therefore, a threat only to persons in the immediate vicinity of the affected patient.

Incubation Period:

Can range from 7-19 days. Typically, onset of illness occurs after 10-14 days and 2-4 more days for onset of rash to occur.

Communicability:

Person-to-person: Yes (high). Approximately 3 weeks (usually corresponds with the initial appearance of skin lesions to their final disappearance; most infectious during the first week of rash via inhalation of virus released from oropharyngeal lesion secretions.

Some infected people shed the virus without ever showing signs of illness.

Survival Outside the Host:

The virus can be propagated in a monkey kidney cell line
Specimens of blood, scrapings from skin lesions, saliva, pustular fluid, and crusts can be transported and stored for short periods without refrigeration. Materials from smallpox patients (dried fluid and crusts) containing virus remain infectious at room

temperature for approximately 1 year. The infectivity of the virus is maintained at 4°C for several months and at -20 to -70°C for years.

Based on the behavior of vaccinia virus, it is believed that aerosolized variola can retain its infectivity for up to 24 hours if not exposed to UV light, and if temperatures are cool (10°C to 11°C) and humidity low (20%). Variola can be almost completely destroyed within 6 hours in an atmosphere of high temperature (31°C to 33°C) and humidity (80%). At cooler temperatures (26°C), variola virus has survived for 8 weeks at high relative humidity and 12 weeks at a relative humidity less than 10%. Virus has been isolated from scabs that had been sitting on a shelf for 13 years.

It was also found to be viable in bread, salad, sausages and gauze bandages stored at 4°C for up to 2 weeks, and in storm water kept at 4.5 °C for up to 166 days.

Samples of freeze-dried virus in a laboratory have been revived after storage for 20 years.

Medical Surveillance:

In the past, smallpox was sometimes confused with chickenpox. The centrifugal distribution of lesions, mostly on the face and extremities than on the trunk, is a distinctive diagnostic feature of smallpox which serves to distinguish it from chickenpox, which is characterized by much more superficial lesions that are concentrated mostly on the trunk, as opposed to the face and extremities.

There are several methods for confirming the diagnosis. Some are specific for variola virus, and others are for *orthopoxviruses* in general. Haemadsorption with susceptible chicken erythrocytes is an early detection method for infection with smallpox virus. Giemsa-stained smears of material from skin lesions may show Guarnieri inclusion bodies. The soluble antigens in blood, vesicle fluid, pustule fluid, and saline extracts from crusts or scrapings in certain stages of disease can be detected via complement fixation, haemagglutination inhibition, immunofluorescence, and Ouchterlony techniques. Serologic response is variable in partially immune patients, who may present clinically with *variola sine eruptione.*

Specimens such as vesicular or pustular fluids or scabs can be examined directly for the presence of virions by electron microscopy, and viral antigen can be identified by immunohistochemical studies. Isolation of the virus in live-cell cultures, followed by PCR, or growth on chorioallantois, is confirmatory; however, PCRdiagnostic techniques are more accurate.

The results of serologic testing do not differentiate among *orthopoxvirus* species, and paired serum samples are required to distinguish recent infection from vaccination in the remote past. Newer methods, that detect IgM responses, may enhance the sensitivity and specificity of serological test.

Other diagnostic methods such as immunodiffusion technique, ELISA, restriction fragment-length polymorphisms, and *in situ* hybridization have been suggested by various laboratories for confirming the presence of *Variola.*

Note: All diagnostic methods are not necessarily available in all countries.

First Aid / Treatment:

If possible, a suspected case of smallpox should be managed in a negative-pressured room, and the patient should be vaccinated, particularly in an early stage of illness. Strict respiratory and contact isolation is imperative. When there are many patients, an isolation hospital or other facility should be designated. Penicillinase-resistance antimicrobial agents should be used if smallpox lesions are secondarily infected, if bacterial infection endangers the eyes, or if the eruption is very dense and widespread. Daily eye rinsing is required in severe cases. Patients need adequate hydration and nutrition. Topical idoxuridine should be considered for the treatment of corneal lesions, although its efficacy is unproved for smallpox.

Leukocyte transfer from immuno-compromised persons, and methisazone have been used to treat *Vaccinia gangrenosa (or progressive vaccinia)*, which is characterized by a slowly progressive enlargement and necrosis or gangrene of the skin at the site of smallpox vaccination that can be fatal if treated with non-specific measures only (antibiotics or steroids alone).

Immunization:

The vaccine consists of a live *Vaccinia virus* , which is a "pox"-type virus related to smallpox. There are significant side effects and risks associated with this vaccine, such as skin complications (eczema vaccinatum) which may occur in people with pre-existing eczema, allergic reactions at site of vaccination, vaccinia gangrenosa (or progressive vaccinia), eye infections (spread of virus from site of vaccination), postvaccinal encephalitis, intrauterine vaccinia, and viremia.

Thus vaccination should be administered only to those exposed to the virus or facing a high probability of exposure.

A successful primary vaccination confers full immunity to smallpox in more than 95% of persons for perhaps 5 to 10 years, and successful revaccination probably provides protection for 10 to 20 years or more.

The recently recognized risk of myopericarditis with both first and second generation vaccinia vaccines serves as a reminder that larger-scale studies of newer vaccines are necessary to further define their safety profiles and relative roles in protection against smallpox.

Two attenuated vaccine strains have also been isolated and tested: modified vaccinia Ankara (MVA), which has been effectively used in more than 1900 people with few adverse effects and a Japanese strain (LC16m8), which is licensed in Japan, and was safely used on more than 50,000 children in the 1970.

Vaccination every 3 years is require by the LRN for the PCR procedure.

Post Exposure Prophylaxis (PEP):

Vaccination very early in the incubation period (within 3 to 4 days of exposure) can markedly attenuate or even prevent clinical manifestations of smallpox. Full protection occurs after successful vaccination.

Vaccination at 4 to 7 days after exposure likely offers some protection from disease or may modify the severity of disease. Unless directly exposed, the vaccine is not advised for groups of people at greater risk for serious side effects, including pregnant and nursing women, children younger than 12 months of age, immuno-compromised patients, HIV patients, or patients with a history of eczema. Vaccinia immune globulin has also been administered (0.6 ml/kg IM) within 3 days of exposure.

Laboratory Facilities:

Containment Recommendations

Containment Level 4 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials, animals, or cultures.

Laboratory Hazards:

Laboratory Acquired Infections:

Except for a laboratory-associated smallpox death at the University of Birmingham, England, in 1978, no further cases have been identified. All known variola virus stocks are held under security at WHO collaborating centers located at Centers for Disease Control and Prevention, Atlanta, United States, or the State Research Centre of Virology and Biotechnology, Koltsovo, Novosibirsk Region, Russia. The possession and use of variola viruses are restricted to the WHO collaborating centers.

Other Precautions

All activities with infectious material should be conducted in a biological safety cabinet (BSC) in combination with a positive pressure suit, or within a class *III* BSC line. Centrifugation of infected materials must be carried out in closed containers placed in sealed safety cups, or in rotors that are loaded or unloaded in a biological safety cabinet. The integrity of positive pressure suits must be routinely checked for leaks. The use of needles, syringes, and other sharp objects should be strictly limited. Open wounds, cuts, scratches, and grazes should be covered with waterproof dressings. Additional precautions should be considered with work involving animal activities.

Sources/Specimens

Scrapings of skin lesions, scab materials, papular, vesicular, or pustular fluid, crusts, bodily fluids including blood and urine, respiratory secretions, pharyngeal and tonsillar swabbings.

Primary Hazards

Ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues.

Special Hazards

Genetically engineered recombinant vaccinia viruses pose a potential risk to laboratory personnel, through direct contact or contact with clinical materials from infected volunteers or animals.

Personal Protective Equipment (PPE)

PPE for Laboratory Personnel:

When handling *suspected Variola major* directly or in a manner in which exposure might occur, the following procedures apply:

1. Wear nitrile gloves
2. Wear eye protection
    a. When working with liquid forms of the *suspected Variola major* a faceshield is to be worn when using a disposable N-95 or P100 filter mask.
    b. When working with dry forms of the *suspected Variola major*
        1) A PAPR that provides eye protection must be worn
3. Respiratory Protection
    a. When working with dry forms of the *suspected Variola major*
    b. A PAPR that provides eye protection must be worn

Decontamination and Disposal

Decontamination

10% acidified household bleach (0.5% sodium hypochlorite)

Disposal

Decontaminate all materials for disposal by steam sterilization, chemical disinfection, and/or incineration.

Storage

In leak-proof containers that are appropriately labelled and locked in a BSL-4 laboratory. LACPHL is a non-Tier 1 laboratory and as such, must destroy or transfer the agent within 7 days of identification.

Agent: Yersinia pestis

Physical Characteristics

Gram negative rod-ovoid 0.5-0.8 µm in width and 1-3 µm in length, bipolar staining (safety pin appearance), facultative intracellular, non-motile

In addition to clinical specimens *Yersinia pestis* can found in

1. A liquid (broth) form
2. Culture form
3. Lyophilized form

Attenuated strains of Yersinia pestis excluded:

Pgm$^-$ mutants of *Yersinia pestis* occur at a high frequency and result in avirulence and Pgm$^-$ strains such as the EV 76 strain have been used for years as live human vaccines with no significant plague-associated problems. The mutation in question is due to the excision of about 102-kb of chromosomal DNA via reciprocal recombination between adjacent IS 100 elements. The lost DNA sequence encodes the ability to synthesize and utilize the siderophore yersiniabactin, which is necessary for growth in mammalian peripheral tissue, as well as the Hms$^+$ locus, which is necessary for biofilm production in the flea vector. However, PCR and/or Southern blot analysis will be required to ensure that "Pgm$^-$" derivatives have undergone this deletion rather than a mutation in the hemin storage genes (*hms*), which also causes loss of Congo red (CR) binding, which is the most common characteristic used to evaluate the pigmentation phenotype.

Strains of *Yersinia pestis* that lack the 75 kb low-calcium response (Lcr) virulence plasmid are excluded. Strains lacking the Lcr plasmid (Lcr$^-$) are irreversibly attenuated due to the loss of a virulence plasmid. An Lcr$^-$ strain of *Yersinia pestis* (Tjiwidej S) has been extensively used as a live vaccine in humans in Java. Thus, these strains pose no significant threat to public health.

Health Hazard Information

Pathogenicity:

Zoonotic disease; bubonic plague with lymphadenitis in nodes receiving drainage from site of flea bite, occurring in lymph nodes and inguinal areas, fever, 50% case fatality if untreated; may progress to septicemic plague with dissemination by blood to meninges; secondary pneumonic plague with pneumonia, mediastinitis, and pleural effusion; untreated pneumonic and septicemic are fatal.

Virulence factors: The V and W antigens and the F1 capsular antigens are expressed only at 37°C and not at the lower (body) temperature of the flea (20 to 25°C).

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. Veterinary staff and pet owners have become infected when handling domestic cats with oropharyngeal or pneumonic plague.

Infectious Dose:

Unknown.

Host Range

Humans, > 200 mammalian species

Mode of Transmission:

Result of human intrusion into zoonotic (sylvatic) cycle or by entry of rodents or infected fleas into human's habitat and bite of infected fleas; domestic pets can carry plague-infected fleas; contact of commensal rodents and their fleas with sylvatic rodents may result in epizootic and epidemic plague; handling of infected tissues; airborne droplets from humans or pets with plague pneumonia; careless manipulation of laboratory cultures; person-to-person transmission by human fleas.

Incubation Period:

From 2 to 6 days; may be a few days longer in vaccinated individuals; for primary plague pneumonia, 1 to 6 days, usually short

Communicability:

Fleas may remain infective for months; bubonic plague not usually transmitted directly from person-to-person; pneumonic plague may be highly communicable under appropriate climatic conditions (overcrowding facilitates transmission)

Medical Surveillance:

Monitor for symptoms; presumptive diagnosis by visualizing bipolar staining, ovoid, gram-negative organisms in sputum or material aspirated from bubo; FA and ELISA test; PHA using Fraction-1 antigen.

Immunization:

Although field trials have not been conducted to determine the efficacy of licensed vaccines, experience has been favorable; immunization is recommended for personnel working regularly with culture of *Y. pestis* or infected rodents, boosters are required every 6 months if high risk continues; protection against pneumonic form is limited.

Prophylaxis:

Chemoprophylaxis using tetracyclines or sulfonamides; for close contacts of pneumonic cases.

Emergency Response to Potential Exposure

Wash hands with soap and water; shower with soap and water.

Laboratory Facilities

Containment Recommendations

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.

Laboratory Hazards:

Laboratory Acquired Infections:

The agent may be present in bubo fluid, blood, sputum, cerebrospinal fluid (CSF), feces, and urine from humans, 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 rodents, infectious aerosols or droplets generated during the manipulation of cultures, and infected tissues. In the necropsy of rodents, primary hazards to laboratory personnel include accidental autoinoculation, ingestion, and bites by infected fleas collected from rodents.

Laboratory Safety

Sources/Specimens:

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:

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 auto-inoculation when handling potentially infected live or dead animals.

Special Hazards:

- Naturally and experimentally infected animals pose a risk to laboratory and animal care personnel.
- Preparing slides, vortexing tubes, or for activities with a high potential for aerosol production, must be placed inside a certified biological safety cabinet (BSC) to minimize exposure to potential aerosols.
- When handling suspected dry forms *Yersinia pestis* two individuals must be present in the room and they must wear respirators equipped with HEPA filters.

Personal Protective Equipment (PPE)

PPE for Laboratory Personnel:

When handling *Yersinia pestis* directly or in a manner in which exposure might occur, the following procedures apply:

1. Wear nitrile gloves
2. Wear eye protection
    a. When working with liquid forms of the *Yersinia pestis* a faceshield is to be worn when using a disposable N-95 or P100 filter mask.
    b. When working with dry forms of the *Yersinia pestis)*
        1) A PAPR that provides eye protection must be worn
3. Respiratory Protection
    a. When working with dry forms of the *Yersinia pestis*
        1) A PAPR that provides eye protection must be worn

PPE for Emergency Responders, Cleanup Personnel, and Investigators

See the “Red Zone” PPE guidance in OSHA’s Anthrax Matrix (http://www.osha.gov/dep/anthrax/anthraxmatrixred.html)

Decontamination

10% acidified household bleach (0.5% sodium hypochlorite)

Storage

LACPHL is a non-Tier 1 laboratory and as such, must destroy or transfer the agent within 7 days of identification.

Training

Training specific to *Yersinia pestis* is required and must be documented for all laboratory personnel working *Yersinia pestis* before starting work with the agent and at intervals thereafter.

D. APPENDIX I

DECONTAMINATION

Materials

1. **Alcohol (e.g., 70% ethanol, isopropanol)**
   a. Use to decontaminate work surfaces only.
2. **Glutaraldehyde or suitable alternative**
   a. Dirty glassware
   b. Equipment decontamination
3. **Chlorine, iodophors, phenolic compounds, quaternary ammonium compounds.**
   a. Work surfaces
   b. Dirty glassware
   c. Equipment decontamination

Procedures

A. Preparation of 10% acidified household bleach (0.5% sodium hypochlorite), working solution

1. Prepare fresh daily.
2. Add one part household bleach to eight parts tap water (5,000 mg of available chlorine per liter) and one part vinegar to acidify to near neutrality (pH 7)
3. Dispense in wash bottles.
4. Record the date prepared on the bottles.
5. Leave wash bottles in the work areas.
6. After 24 h, pour unused bleach solution down the drain and flush the drain with running water to prevent corrosion of pipes.
7. Allow bottles to air dry.

B. Decontamination of work surfaces

1. Follow manufacturer's instructions for cleaning and appropriate disinfecting solutions. Allow bleach solutions to air dry (minimum contact time should be 10 min).
   1. e.g. Biosafety cabinets (stainless steels and glass) use the bleach solution above in A.
2. Use a paper towel or soft cloth soaked with the recommended disinfectant solution.
3. Follow this with 70% alcohol to prevent corrosion of the surface if applicable (e.g. stainless steel).
4. Decontaminate
   1. Before and at the end of the work shift
   2. Upon completion of a procedure
   3. When surfaces become overtly contaminated.

C. Decontamination of equipment (including vortex, centrifuge, and telephone)

A. Follow manufacturer's instructions for cleaning and suitable disinfecting solutions.
B. Use a soft cloth moistened with recommended disinfecting solution.
C. Decontaminate
    1. Upon completion of a procedure
    2. When surfaces become overtly contaminated.
    3. Rinse equipment with tap water (except when alcohol is used).
    4. If the equipment needs to be replaced or serviced, it must be decontaminated before removal.

D. Decontamination of reusable biohazard pail

A. Remove biohazard lining bags and tape shut with white tape.
    1. If a bag is ripped or torn, place it inside a second bag.
    2. Place red biohazard tape on each bag.
    3. Place each bag on a biohazard cart or in a waste dumpster for disposal.
B. Wipe the entire inside surface of the pails with soft cloths soaked with a fresh solution of 10% bleach.
C. Allow a pail to air dry before placing a new biohazard lining bag into it.
D. Inspect each pail for holes or leakage.
E. Decontaminate
    1. Whenever the biohazard lining bag is changed or at least once per week
    2. Whenever a biohazard lining bag is torn

E. Decontamination of computer keyboards

A. Clean and disinfect keyboards at the end of every shift and when the keyboards are visibly contaminated.
B. Unplug the keyboard from the computer.
C. Clean the keyboard surface with a soft cloth moistened with 70% ethanol, or use an alcohol prep pad.
    1. If the keyboard has a fitted covered, inspect the cover for holes or tears.
    2. If the keyboard is uncovered, avoid spilling alcohol underneath the keys.
D. Plug the keyboard back into the computer.
E. If the computer is overtly contaminated with a specimen, clean with a 10% bleach solution and rinse with water.

LIMITATIONS OF THE PROCEDURES

A. Disposable gloves, impervious gowns, eye protection, and masks should be worn when preparing disinfectants and when cleaning and decontaminating soiled equipment.
B. Overtly contaminated reusable equipment and surfaces should be cleaned with an aqueous detergent solution before application of the disinfectant for optimum effectiveness.
C. *Do not* use alcohol solutions for equipment decontamination in poorly ventilated areas or near open flames.
D. *Do not* use glutaraldehyde solutions as general surface disinfectants because of their irritating vapors and prolonged contact time.
E. Inactivation of the causative agent of transmissible spongiform encephalopathies, or prion disease (e.g., Creutzfeldt-Jakob disease), is not achieved by conventional inactivation procedures, heat, irradiation, and chemical germicides. Specimens containing prion proteins include tissues of the central nervous system (high risk); CSF, lymph nodes, spleen, pituitary gland, and tonsils (medium risk); and bone marrow, liver, lungs, thymus, and kidneys (low risk). Blood samples carry no risk of transmitting prion disease.
    1. Avoid the generation of aerosols or droplets during the manipulation of tissues or fluids and during necropsy of experimental animals.
    2. Wear gloves for all activities that skin contact with infectious tissues and fluids may occur.
    3. Use disposable plasticware whenever possible.
    4. Formaldehyde-fixed and paraffin-embedded tissues, especially of the brain, remain infectious (they may be immersed for 30 min in 96% formic acid or phenol before processing, but this may distort microscopic neuropathology or alter immunohistochemical reactions).
    5. Contaminated surfaces should be cleaned and then decontaminated with a 1:10 dilution of sodium hypochlorite.
    6. Contaminated items and medical devices should be decontaminated by either of the following conditions
        a. 134˚C for 18 min in a pre-vacuum sterilizer (liquids must be removed before sterilization)
        b. 121 to 132˚C for 1 h in a gravity displacement sterilizer
        c. Soak in 1 N NaOH or 2.5% NaOCl for 1 h at room temperature.
    g. For reusable instruments, the World Health Organization recommends more stringent guidelines for disinfection (e.g., combined use of 1 N NaOH and autoclaving at 134˚C).

Quality Control

Sign initials in the appropriate box on the decontamination worksheet after completion of each task.

G. APPENDIX II *Quality Assurance of PCR extraction and TRF filtered materials removed from the BSL-3 Suite for testing in BSL-2 areas.*

PURPOSE: This procedure outlines the guidelines for testing materials as a periodic check of adequate removal of bacterial agents prior to removal of items from the BSL-3 suite.

PROCEDURE:

- **a.** Label plate according to labeling protocol.
- **b.** Culture 5 ul of material on agar plates or in liquid broth if necessary.
- **c.** Incubate at appropriate temperature for appropriate length of time.
  - i. *Bacillus anthracis* and *Yersinia pestis* should be incubated per LRN protocols for 72 hours
  - ii. *Francisella tularensis, Brucella species* and multi-agent assay specimens should be incubated per LRN protocols for 5 days.
- **d.** Containers/vials may be wiped down with 10% bleach solution and removed from the laboratory.
- **e.** Check cultures for any signs of growth each day.
- **f.** No bacterial growth should be present after the appropriate incubation period.
- **g.** Sterility testing results can be documented in the laboratory notebook.

COMMENTS:

a. In some instances, such as analysis of official samples, additional analysis is required on samples that have been rendered non-viable. One example is preparation of PCE templates from food samples or bacterial isolates. To expedite sample analysis, methods for rendering organisms non-viable validated. Once these methods are validated, materials rendered non-viable using these methods can be removed from the laboratory without waiting for the maximal growth period.

b. Any changes in the methods will require a validation of the sterility before material can be removed from the laboratory. The method must be repeated successfully a minimum of 10 consecutive times before materials can be removed from the suite using this method without a sterility check. The method must be validated in each food matrix before materials can be removed from the suite in a particular food.

c. The methods should be checked periodically to confirm that they continue to render organisms non-viable.

D. **APPENDIX III** Fever Watch Procedure

Guidelines for fever following possible exposure to an agent such as *Yersinia pestis*, *Francisella tularensis*, *Brucella* species, or *Coxiella burnetii*.

1. Notify the supervisor, safety officer and the R.O. of possible exposure incident.
2. Complete incident report forms as directed
3. Check-out digital thermometer and obtain temperature log sheet.
4. Monitor temperature twice daily AM and PM and record on temperature log sheet.
5. If fever greater than 99º F on two consecutive readings contact physician and describe possible exposure event and identify the agent.
6. Follow doctors instructions
7. Complete workman's compensation forms

I. **APPENDIX IV** Fever Watch Log

Name: _____

Date started working in BTRU with Select Agents: _____

Baseline temperature: _____

Day	Temperature	Time of reading	Initials	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				

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