Safety Training for Biological Threat Agents

APPROPRIATE BIOSAFETY LEVEL (BSL) WORKING CONDITIONS FOR EACH THREAT AGENT/TOXIN:

Agent/Toxin	BSL	Laboratory Risk
B. anthracis	2/3	medium
Y. pestis	2/3	medium
F. tularensis	2/3	high
Brucella spp.	2/3	high
Smallpox	4	high
Burkholderia spp.	3	high
Ricin toxin	2/3	high
Coxiella burnetii	2/3	medium
H5 Avian Influenza	3	high

A. Types of Biological Agents and Toxins

Biological Agents and Toxins include infectious agents of humans, plants, and animals, as well as the toxins that may be produced by microbes and by genetic material potentially hazardous by itself or when introduced into a suitable vector. Biological agents and toxins may exist as purified and concentrated cultures but may also be present in a variety of materials such as body fluids, tissues, soil samples, etc. The agents of biowarfare are primarily those that can be easily transmitted by the airborne route, survive desiccation, and cause serious illness. The specific biological agents and toxins stored/utilized within the BSL-3 area (rooms 1714 & 1714A) and stored in the BT Suite area (room 1715 only) may be found below. Information concerning the specific hazards associated with all registered select agents and toxins as well as the appropriate actions to contain the agents and toxins may be found in the "Infectious Agents Material Safety Data Sheets" manual located in rooms 1714 and 1715.

1. *Bacillus anthracis*

   Agent Specific Information:
   Large Gram positive bacillus that forms non-pigmented colonies; colonies are dry with "ground glass" surface, slightly convex with irregular edges.
   Biosafety Level:
   Level 2- clinical specimens
   Level 3- bacterial cultures
   Storage Condition and Location:
   -80°C, RM 1715
   Select Agent Status:
   Bacillus anthracis (Pasteur strain) - CDC BC3132. **SELECT AGENT; REQUIRES CDC/APHIS SAP FORM 2.** Positive control strain for

the following *B. anthracis* ID test: gamma-phage lysis. Concentration: spore suspension of 2x10E8 colony-forming units per ml. ATCC 4229.

2. *Yersinia pestis*

Agent Specific Information:

Plump Gram negative rod which may exhibit bipolar staining. Colonies appear gray-white to slightly yellow opaque raised, irregular "fried egg" appearance.

Biosafety Level:

Level 2- clinical specimens
Level 3- bacterial cultures

Storage Condition and Location:

-20 to -80°C, RM 1715

Select Agent Status:

Y. pestis avirulent positive strain A1122 is not a select agent. A1122 is equivalent to ATCC 11953.

3. *Francisella tularensis*

Agent Specific Information:

Faintly staining very tiny Gram negative coccobacillus. Colonies appear a gray-white to bluish-gray with entire bordered and smooth, flat surface.

Biosafety Level:

***** HIGHLY INFECTIOUS*****
Level 2- clinical specimens
Level 3- bacterial cultures

Storage Condition and Location:

-20 to -80°C, RM 1715

Select Agent Status:

F. tularensis LVS attenuated positive control strain is not a select agent. **REQUIRES USDA PERMIT.** LVS is equivalent to ATCC 29684.

4. *Brucella spp.*

Agent Specific Information:

Faintly staining Gram negative coccobacillus, appears like "fine sand". Colonies appear pinpoint, smooth, with an entire border and translucent.

Biosafety Level:

***** HIGHLY INFECTIOUS*****
Level 2- clinical specimens
Level 3- bacterial cultures

Storage Condition and Location:

-20 to -80°C, RM 1715

Select Agent Status:

Brucella abortus (Strain 19) – positive growth control for phage strain. Is not a select agent. **REQUIRES USDA PERMIT.** ATCC27565
Brucella abortus (Strain RB51) – positive growth control CO2 enhanced strain. Is not a select agent. **REQUIRES USDA PERMIT.**
Brucella suis (Strain 1330) - CDC BC3170. **SELECT AGENT; REQUIRES CDC/APHIS SAP FORM 2.** Live stock culture of *B. suis* used as a positive growth control. ATCC 23444.

Brucella melitensis (Strain M16)- CDC BC3171. **SELECT AGENT; REQUIRES CDC/APHIS SAP FORM 2.** Live stock culture of *B. melitensis* used as a positive growth control, ATCC 23456. Brucellosis is the most commonly reported laboratory-associated bacterial infection.

5. Ricin toxin (*Ricinus communis* toxin)
   Agent Specific Information:
   Poison that can be made from waste left over from processing castor beans. Can be in the form of a powder, a mist, or a pellet, or it can be dissolved in water or weak acid.
   Biosafety Level:
   Minimum BSL-2 facilities and BSL-3 practices
   Storage Condition and Location:
   2-8°C, RM 1714
   Select Agent Status:
   Ricin toxin, A chain, obtained from Vector Laboratories is not subject to regulation as a Select Toxin since the aggregate amount does not exceed 100 milligrams.
6. *Burkholderia mallei*
   Agent Specific Information:
   Burkholderia mallei is a Gram negative non-motile coccobacillus or slightly curved rod with rounded ends. *B. mallei* can appear singly, in pairs end-to-end, or parallel bundles. Colonies appear as smooth, gray, translucent at 48 hrs on SBA.
   Biosafety Level:
   **** HIGHLY INFECTIOUS****
   Level 3- bacterial cultures & clinical specimens
   Storage Condition and Location:
   Tier 1 SELECT AGENT – no longer in entity's possession
   Select Agent Status:
   Burkholderia mallei: **SELECT AGENT; REQUIRES CDC/APHIS SAP FORM 2.** Positive control strain for presumptive and confirmatory LRN procedures. ATCC 23344.
7. *Burkholderia pseudomallei*
   Agent Specific Information:
   Burkholderia pseudomallei is a motile Gram negative aerobic bacillus. Colonies vary from smooth to wrinkled; on SBA the colonies are smooth and creamy at 24 hrs but may become dry and wrinkled at 48-72 hrs.
   Biosafety Level:
   **** HIGHLY INFECTIOUS****
   Level 3- bacterial cultures & clinical specimens
   Storage Condition and Location:
   Tier 1 SELECT AGENT – no longer in entity's possession
   Select Agent Status:
   Burkholderia pseudomallei: **SELECT AGENT; REQUIRES CDC/APHIS SAP FORM 2.** Positive control strain for presumptive and confirmatory LRN procedures. ATCC 23343.
8. *Coxiella burnetii*
   Agent Specific Information

Q fever is a zoonotic disease caused by *Coxiella burnetii,* a species of bacteria that is distributed globally. Many human infections are unapparent. *Coxiella burnetii* is a highly infectious agent that is rather resistant to heat and drying. It can become airborne and inhaled by humans. A single *C. burnetii* organism may cause disease in a susceptible person. This agent could be developed for use in biological warfare and is considered a potential terrorist threat.

Biosafety Level:

Level 2- Clinical specimens

Level 3- Bacterial Isolates

Storage Condition and Location:

No isolates at this lab for control use or otherwise.

Select Agent Status:

Coxiella burnetii isolates are currently not in laboratory use. **Coxiella burnetii is listed as a select agent. REQUIRES CDC/APHIS SAP FORM 2.** Real-time PCR is used for detection and presumptive identification.

9. H5 Avian Influenza Virus

Agent Specific Information:

An influenza A virus subtype that occurs mainly in birds. A few cases of human-to-human spread of H5N1 have occurred.

Biosafety Level:

Level 3 – Clinical specimens

Storage Condition and Location:

-80^{o}C, RM 1715

Select Agent Status:

Inactivated, noninfectious positive control, vaccine candidate virus, generated by reverse genetics. Contains human cell material (for RNase P). This control is not a Select Agent.

COMPLIANCE METHODS

A. Universal precautions will be observed in order to prevent contact with specimens or other potentially infectious materials/toxins. All specimens or other potentially infectious material will be considered infectious regardless of the perceived status of the source. All cuts or open sores must be bandaged at all times even if wearing gloves.

B. Universal precautions consist of the following points:
   1. Routinely use barrier protection to prevent skin and mucous membrane contamination with all specimens or other potentially infectious materials/toxins.
   2. Wear gloves when:
      a. Touching specimens or other potentially infectious materials/toxins, including during routine laboratory work.
      b. Touching all laboratory specimens and tissues.
      c. Handling items contaminated with potentially infectious materials/toxins, including specimen containers, laboratory

instruments, countertops, etc. (Observe clean technique, i.e. avoid cross-contamination.)

3. Wear a mask and eye covering (safety glasses), or preferably a face shield, during procedures that are likely to generate droplets of potentially infectious materials/toxins to prevent exposure of the mucous membranes of the mouth, nose and eyes. Eye and face protection must either be disposed of with other contaminated laboratory waste or decontaminated before use. Persons who wear contact lenses in laboratories must also wear eye protection.
4. Wash hands or other skin surfaces thoroughly and immediately if contaminated with specimens or other potentially infectious materials/toxins.
5. Change gloves frequently.
6. Wash hands immediately after gloves are removed.
7. Take extraordinary care to avoid accidental injuries caused by needles, scalpel blades, laboratory instruments, etc. when performing procedures, cleaning instruments, handling sharp instruments, and disposing of used needles. Broken glassware and other sharp items may be removed only by using a mechanical device or tool (forceps, tongs, broom, and dust pan).
8. Place used needles, skin lances, scalpel blades, and other sharp items into a puncture-resistant biohazard container for disposal. The container should be located as close as possible to the work area.
9. To prevent needlestick injuries, needles must not be recapped, purposely bent, cut, broken, removed from disposable syringes or otherwise manipulated by hand.
10. Any non-disposable sharps must be disinfected with a 1:10 solution of 5.25% - 6.15% hypochlorite solution (5250-6150 mg/L) made fresh daily or Bleach Germicidal Cleaner disinfectant. If used within the BSL-3 laboratory, they must also be placed in an autoclavable sterility pouch which must be placed in a hard-walled container and transported to the autoclave room for steam sterilization.
11. Minimize the need for mouth-to mouth emergency resuscitation procedures. Mouthpieces, resuscitation bags, or other ventilation devices should be used routinely.
12. Take care to minimize the formation of droplets, spatters, splashes, and spills of specimens or other potentially infectious materials/toxins.
13. Work surfaces are decontaminated at least once a day and after any spill of viable material. See Section IV.D Disinfection/ Decontamination for instructions.

C. All employees with exudative lesions or weeping dermatitis should refrain from handling contaminated equipment and specimens until the condition resolves. NOTE: Alternatively, skin lesions should be covered with an occlusive bandage to prevent contamination.

D. Pregnant women are not known to be at greater risk of contracting bloodborne infections than other laboratory workers. However, pregnant employees should be especially aware of universal precautions.

E. Biosafety training for all laboratory personnel on Biosafety Level 2 and Biosafety Level 3 practices for all potentially infectious materials/toxins, as well as proper use of all Personal Protective Equipment (PPE), will occur on an annual basis. New employees will receive biosafety training immediately after hire. Personnel must receive annual updates or additional training when procedural or policy changes occur.

F. Visitors entering the Biosafety Level 3 laboratory area (rooms 1714A and 1714) and rooms 1715-1718 must undergo biosafety training on an annual basis before entering these areas. There are training materials located outside each of the areas containing agent/toxin information, compliance methods, engineering and work practice controls and decontamination procedures. A training log is located outside each of the areas and must be signed indicating that training was received.

G. The Laboratory Director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements and who comply with all entry and exit procedures enter the areas where select agents/toxins are stored/used.
   1. Biosafety training for select agents/toxins is required for all visitors entering the areas where those items are stored/used. All visitors will sign a log indicating that they have received this training annually.

H. Immunizations for certain agents handled or potentially present in the laboratory are available for laboratory personnel. The Hepatitis B vaccine is available for all personnel. The smallpox vaccination is available for personnel working within the BSL-3 facility with the risk of exposure to smallpox. The anthrax vaccination is available for those with a risk of exposure to anthrax.

ENGINEERING AND WORK PRACTICE CONTROLS

A. Engineering and work practice controls will be utilized to eliminate or minimize exposure.
   1. Employees must wash their hands or other skin with soap and water, or flush mucous membranes with water, as soon as possible following an exposure incident (such as a splash of blood to the eyes or an accidental needle stick.)
   2. Employees must wash their hands immediately (or as soon as feasible) after removal of gloves or other personal protective equipment. Hands should be scrubbed vigorously for at least 20 seconds with anti-microbial soap or alcohol based hand rub; dry hands with disposable towels. Additional scrub time and reapplication of soap/rub may be performed, and a liberal soaking with 70% isopropyl alcohol may be

used in advance of washing hands if probability of skin contamination is high. Hand washing protocols must be rigorously followed.

3. Employees who encounter improperly disposed needles shall notify their supervisor or manager of the location of the needle(s). Additionally, the medical director and other appropriate authorities at the site shall be notified. Needles shall be disposed of in labeled sharps containers provided at each location.
    a. Needles must never be recapped.
    b. Needles may be removed only by using a mechanical device or tool (forceps, pliers, broom, and dust pan).
4. In work areas where there is a reasonable likelihood of exposure to potentially infectious materials/toxins, employees are not to eat, drink, apply cosmetics or lip balm, smoke, or handle contact lenses. Food and beverages are not to be kept in refrigerators, freezers, shelves, cabinets, or on counter tops or bench tops where blood or other potentially infectious materials/toxins are present.
5. Mouth pipetting/suctioning of specimens or other potentially infectious materials/toxins is prohibited.
6. All procedures will be conducted in a manner that will minimize splashing, spraying, and generation of droplets of specimens or other potentially infectious materials/toxins.
7. Wear laboratory coat, with long cuffed sleeves, and gloves, covering cuff of sleeves, when handling specimens or potentially infectious materials/toxins. Coats are worn at all times in the laboratory and are not to be worn outside the laboratory work area. Disposable lab coats or gowns are recommended.
8. Double gloves are not necessary if gloves are removed after each use. However, double gloves are required when working within the BSL-3 laboratory. (See “Procedure for the Proper Personal Protective Equipment (PPE) Required for the BSL-3 Laboratory”.) Gloves should not be washed or treated with disinfectants/soap. They should be changed as is necessary/appropriate. Remove and dispose of gloves into proper biohazard waste container for sterilization. Gloves must not be worn outside the laboratory.
9. When handling dry forms of toxins that are electrostatic, do not wear gloves (such as latex) that help to generate static electricity.
10. Due to the fact that the bioterrorism section works with agents that pose a risk to employees as well as to the environment, a gloves risk assessment study was conducted. This evaluation was done on six different types of gloves that were submitted, including latex, vinyl and nitrile, in order to choose the most appropriate kind for use in this laboratory Based on our findings, we concluded that we will use the SensiCare powder-free nitrile brand as well as the SensiCare Ice with Smart Guard powder-free nitrile brand. This brand has performed the best in terms of durability, integrity, grip and fit.
11. The use of respiratory protection and eye protection is required when handling specimens or potentially infectious materials/toxins inside the BSL-3 since there may be a potential for aerosols. Safety glasses, face

shields or other splatter guard devices must be used as a means of protection against anticipated splashes or sprays of infectious or other hazardous materials. Personnel wearing contact lenses must also wear eye protection. Eye and face protection must be decontaminated before reuse or disposed of with other contaminated laboratory waste. Make every possible effort to utilize the BSCs when handling and processing potentially infectious samples/specimens. The respirators of choice for this facility are N-95 masks or Powered Air Purifying Respirators (PAPRs). The PAPRs provide high level protection and consist of a fully enclosed hood with a face shield, PAPR assembly, filtering device, and protective coveralls. Respirator users require annual certification/training.

12. When conducting liquid transfers and other operations that pose a potential splash or droplet hazard in an open-fronted hood or BSC, safety glasses and disposable facemask, or a face shield, must be worn.
13. Use equipment and supplies that maximize containment of suspect materials (e.g., aerosol proof centrifuges, specimen carriers, and centrifuge cups with tight seal). Seals on centrifuges should be checked monthly and replaced if needed. When working with infectious agents inside the BSL-3 laboratory, the centrifuge rotors containing those infectious agents should be removed from the centrifuge and transported to the BSC before opening.
14. Glassware should be replaced with plastic wherever practical to minimize the risk of cuts and abrasions from contaminated surfaces.
15. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
16. All cultures, stocks, and other regulated wastes are decontaminated before disposal by autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak proof container, and closed for transport from the laboratory. Infectious waste from the BSL-3 laboratory should be decontaminated before removal for off-site disposal.
17. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
18. Packaging containers in contact with infectious materials shall be decontaminated or considered a biohazard and be disposed of accordingly. All equipment in contact with infectious materials shall be decontaminated, as appropriate.
19. All open manipulations involving infectious materials/toxins are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

20. Clean-up is facilitated by using plastic-backed paper toweling on non-perforated work surfaces within biological safety cabinets.
21. Protective laboratory clothing such as solid-front gowns, wrap-around gowns, and disposable scrub suits or coveralls are worn by laboratory personnel while in the BSL-3 laboratory. Protective clothing is not worn outside the laboratory. Clothing must be disposed of when overtly contaminated. Disposable scrub suits are available and may be used if other clothing becomes contaminated.
22. A "Fever Watch Log" is included as part of medical surveillance. All employees working within the BSL-3 laboratory will document each time they are working with infectious agents/toxins. If they should become ill, there will be a record of what they could have potentially been exposed to.
23. Since occupational exposure to human pathogens is a risk, baseline serum samples are collected as appropriate and stored for all laboratory and other at-risk personnel. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory.
24. All laboratory personnel manipulating select agents and/or toxins under BSL-3 conditions, including women of child-bearing age, pregnant women, and immunocompromised individuals who may be exposed to human pathogens specific to select agents and toxins are provided with information regarding the hazards they may encounter while working with these materials. This information is conveyed in the *Safety Training for Biological Threat Agents* annual training for those employees. They are provided with Safety Data Sheets (SDS) for each infectious agent and toxin approved for use in this laboratory. First aid/treatment procedures are also included in the SDS for infectious agents. Those individuals with the conditions noted above are encouraged to self-identify to the county's healthcare provider (Concentra) for appropriate counseling and guidance.
25. All high risk operations will be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.
26. Animal testing is not performed at the Tarrant County Public Health Department bioterrorism laboratory, therefore, no animals of any kind will be allowed in the clinical laboratory at any time.
27. Testing involving plants is not performed at the Tarrant County Public Health Department bioterrorism laboratory and no plants of any kind will be allowed in the Biosafety Level 3 laboratory area (rooms 1714A and 1714) and rooms 1715-1718.
28. The biosafety and containment procedures must be sufficient to contain the select agent or toxin (e.g., physical structure and features of the entity, and operational and procedural safeguards). Provisions to assure proper biosafety cabinet performance and air system operation must be verified. The interlock will be tested as part of the annual biological safety cabinet certification, along with certification of the HEPA filters.

29. Movement of BT personnel transporting select agents from registered areas through non-registered areas such as hallways/corridors will occur due to the design of the laboratory. When this is necessary, select agents are secured inside a sturdy hard plastic sealed container to prevent any accidental releases from occurring.
30. Viable select agents are not manipulated outside of the BSL-3 laboratory. Rendering select agents non-viable is achieved by following the directions in the procedure *Inactivation of Select Agents*, such as extraction followed by filtration. This procedure may be found in the *Laboratory Operations Procedures/Policies, Bioterrorism Section* manual. The procedure states that any viable cells (vegetative and spores) are removed after performing this procedure. A sterility check is performed by using 10% of the filtrate incubated on a sheep blood agar plate for a minimum of five days. If the extract is made from *Francisella tularensis*, a chocolate (CHOC) plate must be used instead of SBA.
31. Investigate to determine the reason for any failure of a validated inactivation procedure or any failure to remove viable select agent from material. If the Responsible Official is unable to determine the cause of a deviation from a validated inactivation procedure or a viable select agent removal method, or receives a report of any inactivation failure after the movement of material to another location, the Responsible Official must report immediately by telephone or email the inactivation or viable agent removal method failure to CDC or APHIS.
32. Review, and revise as necessary, each of the entity's validated inactivation procedures or viable agent removal methods. The review must be conducted annually or after any change in Principal Investigator, change in the validated inactivation procedure or viable select agent removal method, or failure of the validated inactivation procedure or viable select agent removal method. The review must be documented and training must be conducted if there are any changes to the validated inactivation procedure, viable select agent removal method, or viability testing protocol.
33. No manipulation of select agents and toxins are allowed at the same time as non-select agents and toxins. The BSL-3 area only has one biological safety cabinet but it is thoroughly decontaminated before and after manipulation of each sample type to prevent unintentional contamination.

B. Special engineering and work practice controls must be utilized when working with or manipulating toxins.
    1. All unrelated and nonessential work should be restricted from areas where stock solutions of toxin or organisms producing toxin are used. When toxins are in use, the BSL-3 should be clearly posted: "Toxins in Use – Authorized Personnel Only".
    2. If toxins are stored in the laboratory, all containers should be sealed, labeled, and secured to ensure restricted access; refrigerators and other

storage containers should be clearly labeled and provide contact information for trained, responsible laboratory staff.

3. All pressurized tubes or other containers holding toxins should be opened in a BSC, chemical fume hood, or other ventilated enclosure.
4. Operations that expose toxin solutions to vacuum or pressure, for example sterilization of toxin solutions by membrane filtration, should always be handled in this manner, and the operator should also use appropriate respiratory protection.
5. Glassware should be replaced with plastic for handling toxin solutions wherever practical to minimize the risk of cuts or abrasions from contaminated surfaces.
6. Thinwalled glass equipment should be completely avoided. Glass Pasteur pipettes are particularly dangerous for transferring toxin solutions and should be replaced with disposable plastic pipettes. Glass chromatography columns under pressure must be enclosed within a plastic water jacket or other secondary container.

C. Risk assessments:

1. A risk assessment must be performed each time a select agent or toxin may be present during a testing event. The risk assessment must encompass the hazards that personnel may encounter. Many variables are present that must be considered for each individual. All biosafety precautions regarding select agents or toxins must be followed. Personnel working with select agents or toxins must be assessed and evaluated if any symptoms of exposure appear. Following testing, inactivation and decontamination would be performed according to the specific instructions. Each assessment performed would be specific to the variables encountered for each select agent or toxin present. The risk assessments that have been created for select agents and toxins are agent-specific biosafety assessments, confirmatory methods assessments, culture methods for preliminary ID assessments and sample receipt, processing, PCR and TRF assessments. These risk assessments may be found in the *Risk Assessments for the Bioterrorism Response and Emerging Agents (BREA) Section* manual.
2. The specific biological agents and toxins stored/utilized within the BSL-3 area (rooms 1714 & 1714A) and stored in the BT Suite area (room 1715 only) may be found in the *Incident Response Plan,* located in the *Laboratory Operations Procedures/Policies for the Bioterrorism Section* manual, Section IV.F.1.b, *Types of Biological Agents and Toxins.*

D. Disinfection/decontamination

1. General decontamination information
   a. Laboratory coats and gloves should always be utilized when cleaning up a spill. Eye and respiratory protection are required if there is any potential for generation of aerosols and/or chemical fumes.
2. Routine decontamination of laboratory work surfaces, **NO** suspect agent

a. Work surfaces should be wiped down both prior to and after use.
b. Decontaminate work surfaces with a 1:10 solution of 5.25% - 6.15% hypochlorite solution (5250-6150 mg/L) made fresh daily or Bleach Germicidal Cleaner disinfectant.

3. Decontamination of *laboratory work surfaces* **WITH** suspect agent
   a. If decontaminating surfaces with toxins and/or spores, decontaminate with a 1:10 solution of 5.25% - 6.15% hypochlorite solution (5250-6150 mg/L) made fresh daily or Bleach Germicidal Cleaner disinfectant.
   b. After allowing to air dry, surfaces may be wiped down with RNase Away.
   c. Dispose of all adsorbent towels/material into autoclave containers/bags for sterilization (60 min, 121^{o} C, 15 psi, slow exhaust).
4. Decontamination of *spills* **WITH** suspect agent
   a. Immediately alert co-workers within close proximity.
   b. Remove gloves and boot covers. Don new PPE (gloves and boot covers)
   c. Gather spill kit and hang spill signs located in spill kit.
   d. Establish spill parameter.
   e. Soak towels located in spill kit with a 1:10 solution of 5.25% - 6.15% hypochlorite, or EPA registered equivalent (Bleach Germicidal Cleaner disinfectant).
   f. Working outside-in, cover spill with towels.
   g. Allow for appropriate contact time. For biological agents, the contact time should be 20-30 minutes, for toxins, 30 minutes. At lower temperatures and/or with significant quantities of organic matter, the contact time may need to be increased (e.g., up to 60 min with 1:10 hypochlorite).
   h. Working outside-in, pick up towels using tongs, also located in spill kit.
   i. Dispose of contaminated towels and waste in biohazard bags/boxes to be autoclaved.
   j. Mop spill area with a 1:10 solution of 5.25% - 6.15% hypochlorite, or EPA registered equivalent (Bleach Germicidal Cleaner disinfectant).
   k. Remove gloves and boot covers. Don new PPE.
   l. Log and report incident. All releases/exposures to select agents or toxins should be immediately reported to CDC by submission of APHIS/CDC Form 3 – Report of Theft, Loss, or Release of Select Agents and Toxins. This form may be found online at http://www.selectagents.gov using the forms tab located near the top of the page.
5. Gross decontamination of BSL-3
   a. If gross contamination of the BSL-3 lab area should occur, vapor phase hydrogen peroxide decontamination must be performed. This is done using the Six Log Phileas Model 501 vaporizer.
6. Decontamination of supplies/waste

a. Contaminated items such as pipettes, needles, loops, and microscope slides should be immersed in decontamination solution until autoclaving.
b. Disposable, microbiological and non-corrosive chemical wastes are decontaminated by autoclaving (60 min, 121^{o} C, 15 psi, slow exhaust) in closed containers/biohazard bags, then labeled properly and disposed of per laboratory biosafety policies.

7. Decontamination of equipment:
   a. Follow published guidelines for decontamination of equipment.
   b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Special decontamination procedure for *Coxiella burnetii*
   a. Special decontamination procedures are necessary for *Coxiella burnetii* because sodium hypochlorite (household bleach) solutions are not completely effective.
   b. Two disinfectants effective against *C. burnetii* are 70% ethanol and 5% Micro-Chem Plus™, a blend of two quaternary ammonium compounds.

E. Emergencies inside the BSL-3 area

Exiting the BSL-3 in an EMERGENCY situation:

1. Non-Life-Threatening Emergency
   a. Examples include minor medical emergencies such as a needlestick injury, HVAC failure or malfunction, bomb threat or power failure.
   b. Alert co-workers and notify supervisor if possible. Important phone numbers are posted near the phone.
   c. Secure select agents.
   d. Exit expeditiously, discarding PPE into receptacle as you leave the anteroom.
   e. Wash hands.
   f. Notify supervisor and RO/ARO.
   g. Seek first aid or request assistance by dialing 9-1-1 for first responders.
   h. Log and report the incident.
2. Life-threatening medical emergency
   a. Examples include major medical emergencies such as heart attack or unconscious individual.
   b. Immediately notify a SRA cleared individual for emergency response assistance or dial 9-1-1.
   c. Assist or remove the injured technician from the BSL-3 lab into the anteroom. Tools for evacuation assistance are located in the anteroom and include the fire blanket and Outside Doffing Kit.
   d. When evacuating in a life-threatening situation, you do not need to remove PPE before exiting the laboratory.
   e. If possible, decontaminate the injured technician and yourself in the anteroom.

f. If possible, remove the PPE from the injured individual and yourself. Use scissors if necessary and discard into receptacle.
g. If doffing must occur outside either the anteroom or the building, take the Outside Doffing Kit upon exit.
h. Remove the injured individual to the anteroom or just outside of the exit and wait for first responders to arrive.
i. Doff normally and discard PPE into biohazardous waste bag from Outside Doffing Kit. All discarded material will be autoclaved before disposal.
j. Use soap and water or hand sanitizer to wash hands after doffing.
k. Notify appropriate staff.
l. If a spill occurred, hang spill signs at the spill perimeter to notify others not to enter.
m. If the exit route was contaminated, PPE from the hallway will be donned and area VHP decontamination will be used prior to returning the area to service.
n. If a spill has occurred in the BSL-3 lab, standard cleanup procedures will be followed prior to returning the area to service.
o. Log the incident.

3. Life-threatening emergency
   a. Examples include man-made events such as building security breached by a car crash, seeing fire or smoke, smelling a gas leak or smoke.
   b. Immediately exit the BSL-3 suite and take the Outside Doffing Kit.
   c. When evacuating in a life-threatening situation, you do not need to remove PPE before exiting the laboratory.
   d. Proceed to designated gathering area near the flagpole. Take extra care to segregate yourself from the rest of the laboratory personnel.
   e. Doff normally and discard PPE into biohazardous waste bag from Outside Doffing Kit. All discarded material will be autoclaved before disposal.
   f. Use soap and water or hand sanitizer to wash hands after doffing.
   g. If a spill occurred, hang spill signs at the spill perimeter to notify others not to enter.
   h. If the exit route was contaminated, PPE from the hallway will be donned and area VHP decontamination will be used prior to returning the area to service.
   i. If a spill has occurred in the BSL-3 lab, standard cleanup procedures will be followed prior to returning the area to service.
   j. Notify the appropriate staff that you have exited the building.
   k. Log the incident.
4. Shelter in place emergency
   a. Examples include tornados and severe weather.

b. Exit expeditiously, discarding PPE into receptacle as you leave the anteroom.
c. Wash hands.
d. Immediately exit the BSL-3 and proceed to the designated lab shelter area.
e. Wait there until the all clear has been given.

SPECIAL PROVISIONS FOR WORKING WITH TOXINS OF BIOLOGICAL ORIGIN

In addition to the general safety guidelines mentioned in the Standard Operating Procedures and throughout the Plan, special precautions are needed when handling toxins of biological origin. A minimum set of guidelines that should be followed is listed below. The lab supervisor should ensure that these and other precautions designed to minimize risk of exposure to these substances are taken.

- Training specific to the toxin(s) used is required and documented for all laboratory personnel working with toxins, before starting work with the toxin and at refresher training annually thereafter.
- An inventory control system is in place.
- Toxins are stored in a locked freezer or refrigerator when not in use.
- Access to areas containing toxins is restricted to those whose have prior DOJ approval.
- Preparation of primary containers of toxin stock solutions and manipulations of primary containers of dry forms of toxins will be conducted in a biological safety cabinet containment system approved by the safety officer. HEPA and/or charcoal filtration of the exhaust air may be required, depending on the toxin.
- The user will verify inward airflow of the hood or biological safety cabinet before initiating work.
- All work will be done within the operationally effective zone of the hood or biological safety cabinet.
- When toxins are in use, the room will be posted to indicate "Toxins in Use - Authorized Personnel Only." Any special entry requirements are posted on the entrance to the room. Only personnel whose presence is required are permitted in the room while toxins are in use.
- All high risk operations will be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.
- Before containers are removed from the cabinet, the exterior of the closed primary container will be decontaminated and placed in a clean secondary container. Toxins will be transported only in leak/spill-proof secondary containers.
- Contaminated and potentially contaminated protective clothing and equipment will be decontaminated using methods known to be effective against the toxin before removal from the laboratory for disposal, cleaning or repair. If decontamination is not possible/practical, materials (e.g., used gloves) will be disposed of as toxic waste. Materials contaminated with infectious agents as

well as toxins will also be autoclaved or otherwise rendered non-infectious before leaving the laboratory.
- The interior of the cabinet will be decontaminated periodically. Until decontaminated, the cabinet will be posted to indicate that toxins are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.

Safety Equipment

- When using an open-fronted biological safety cabinet, protective clothing, including gloves and a disposable long-sleeved gown, will be worn so that hands and arms are completely covered.
- Eye protection will be worn while working with toxins.
- Other protective equipment may be required, depending on the characteristics of the toxin and the containment system. Use additional respiratory protection if aerosols may be generated. If both substitution and engineering controls are unavailable, the use of personal protective equipment may be required to reduce inhalation exposures. Respiratory protection from N-95 masks to self-contained breathing apparatus may be utilized to this end. If laboratory employees wear respirators, requirements of the OSHA Respirator Standard (1910.139) are implemented. This Standard requires training in the proper use of respirators, medical surveillance to ensure the user is capable of wearing a respirator, and fit testing to ensure that the respirator fits properly. A lab worker or his/her supervisor should contact the Laboratory Safety Officer in the event that respiratory protection is utilized to control exposures to hazardous chemicals and toxins. See the Powered Air Purifying Respirator (PAPR) System Procedure in the BT Laboratory Operations Procedures/Policies Manual.
- When handling dry forms of toxins that are electrostatic:
  - Do not wear gloves (such as latex) that help to generate static electricity
- When handling toxins that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic from dermal exposure), double gloves must be worn.
- Consider both toxin and diluent when selecting gloves and other protective clothing.
- If infectious agents and toxins are used together, consider both when selecting protective clothing and equipment.

Spill Procedure

- For spills involving toxins of biological origin, the surfaces involved should be cleaned with 0.1% or 0.5% solution of sodium hypochlorite (0.1% = 1 part household bleach to 49 parts of water; 0.5% = 1 part household bleach to 9 parts of water). No guidelines for contact time are available so post-cleaning surface sampling is recommended to check on effectiveness.

PRECAUTIONS FOR HANDLING SAMPLES FOR VACCINIA, VARICELLA, OR VARIOLA MAJOR

The level of precautions to be used in handling clinical specimens should be consistent with the level of risk of smallpox associated with the patient. Refer to the Algorithm document posted with the poxvirus testing protocols on the LRN website. Compare the documentation associated with the specimen to the criteria outlined in the algorithm.

NOTE: Local laboratories must not attempt to undertake isolation or identification of Variola virus.

a. High risk

- Only personnel successfully vaccinated recently (within 3 years) wearing appropriate barrier protection (gloves, gown, and shoe covers) should be involved in specimen collection for suspected cases of smallpox. Respiratory protection is not needed for personnel with recent, successful vaccination. Masks and eyewear or face shields should be used if splashing is anticipated.
- If unvaccinated personnel must be utilized to collect specimens, only those without contraindications to vaccination should be utilized as they would require immediate vaccination if the diagnosis of smallpox is confirmed. Fit-tested N95 masks should be worn by unvaccinated individuals caring for suspect cases.
- All procedures for obtaining, processing, packing and shipping potentially infectious materials should be performed using BSL-2 or, if available, BSL-3 practices.
- While working with specimens, personnel should avoid any activity that brings hands or fingers in contact with mucosal surfaces, such as eating, drinking, smoking, or applying make-up.
- Upon removal of gloves, personnel should thoroughly wash their hands with soap containing Lysol or soaps such as Hibiclens before leaving the laboratory. Areas of the skin known or suspected to have come in contact with virus/specimen should be washed with soap. If possible, skin should be decontaminated with a 0.5% sodium hypochlorite solution with at least a 1-min contact time.

  After specimen collection is completed, all protective materials worn by the specimen collector (e.g., gloves, mask, gown, shoe covers) and all non-reusable sample collection materials and equipment (e.g., needles, tubes, swabs) must be double bagged in biohazard bags and autoclaved or incinerated before disposal.

b. Moderate risk:

Moderate risk specimens should be handled with the same precautions as high risk specimens.

c. Low risk:

Low risk specimens, including those specimens associated with a known vaccination or associated with a known poxvirus (non-smallpox) outbreak, should be handled with the following precautions

- All procedures for obtaining, processing, packing and shipping potentially infectious materials should be performed using BSL-2 or, if available, BSL-3 practices.
- While working with specimens, personnel should avoid any activity that brings hands or fingers in contact with mucosal surfaces, such as eating, drinking, smoking, or applying make-up.
- Upon removal of gloves, personnel should thoroughly wash their hands with soap containing Lysol or soaps such as Hibiclens before leaving the laboratory. Areas of the skin known or suspected to have come incontact with virus should be washed with soap. If possible, skin should be decontaminated with a 0.5% sodium hypochlorite solution with at least a 1-min contact time.
- After specimen collection is completed, all non-reusable protective materials worn by the specimen collector (e.g., gloves, mask, gown, shoe covers) and sample collection materials and equipment (e.g., tubes, swabs) must be placed in biohazard bags for disposal with other medical waste. Reusable equipment (e.g., goggles, face shield) should be decontaminated and set aside for reprocessing. Needles and other sharp instruments should be placed in the appropriate sharps container.

d. Unknown risk:

If sufficient information is not available to make a determination of the risk associated with the specimen, the specimen should be treated as high risk.

NOTE: These precautions are intended for the protection of personnel performing specimen collection, processing, packaging and shipping. In addition to these precautions, follow your facility's protocols and guidance with regard to appropriate infection control activities.

Specimen storage and transport

1. High or moderate risk specimens

a. Place specimens from a single patient into a biohazard bag with an outside label that includes:
   - Patient name
   - Date of collection
   - Social security number or date of birth of patient

   It cannot be overemphasized that each patient's specimens should be packaged separately from those of other patients to avoid cross contamination.

b. Package specimens from a single patient on refrigerated (2-8^{o}C) gel packs.

c. Formalin-fixed biopsies and EM grids should be shipped at room temperature or with refrigerated specimens. DO NOT FREEZE formalin fixed biopsy specimens or EM grids.

d. Specimens may be stored in conditions outlined above if transported within 24 hours of collection. If this is not possible, store samples on dry ice or at –20^{o}C to –70^{o}C, EXCEPT for electron microscope grids, serum,

and formalin fixed tissue. Serum should remain at 2-8°C. DO NOT FREEZE formalin-fixed biopsy specimens or EM grids.

e. Approval must be obtained from CDC before shipping high or moderate risk specimens. Please refer to Guide D in the Smallpox Response Plan (http://www.bt.cdc.gov/agent/smallpox/response-plan/index.asp) for guidance on obtaining further direction and approval from CDC.

2. Low risk specimens

a. Place specimens from a single patient into a biohazard bag with an outside label that includes:
   - Patient name
   - Date of collection
   - Social security number or date of birth of patient

   It cannot be overemphasized that each patient's specimens should be packaged separately from those of other patients to avoid cross contamination.

b. Package specimens from a single patient (except biopsies) on refrigerated (2-8°C) gel packs.

c. Formalin-fixed biopsies and EM grids should be shipped at room temperature or with refrigerated specimens. DO NOT FREEZE formalin fixed biopsy specimens or EM grids.

d. Specimens may be stored in conditions outlined above if transported within 24 hours of collection. If this is not possible, store samples on dry ice or at –20°C to –70°C, EXCEPT for electron microscope grids, serum, and formalin fixed tissue. Serum should remain at 2-8°C. DO NOT
   FREEZE formalin-fixed biopsy specimens or EM grids.

e. Refer to Laboratory Procedures for Packaging and Shipping Infectious Substances and Biological Agents on the LRN website for detailed instructions.