SENTINEL LEVEL CLINCAL LABORATORY GUIDELINES

FOR

SUSPECTED AGENTS OF BIOTERRORISM

AND

EMERGING INFECTIOUS DISEASES

Clinical Laboratory Bioterrorism Readiness Plan

American Society for Microbiology (ASM)

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INTRODUCTION

The purpose of this template is to provide a model for laboratories to use for developing a bioterrorism (BT) preparedness plan. The components of this template can be used to develop a readiness plan to meet the needs of the institution. It is not meant to be all-inclusive. Rather, it is to serve as an aid in the process of developing a specific plan for each institution.

The laboratory BT preparedness plan should be integrated into the institutional BT preparedness plan.

Some of the specific laboratory protocols for the BT agents included in this template contain flowcharts. Ideally, these flowcharts should be integrated into laboratory procedures so that technologists have ready access to this information.

NOTE: It is quite possible that the laboratory will not be contacted in advance and informed that one of the potential agents of bioterrorism is suspected. As a result, it is essential that appropriate safeguards be taken, including subculture of all blood cultures in a biosafety cabinet or behind a safety shield, following the flowcharts for suspicious agents, and always considering the possibility of bioterrorist agents.

Table of Contents: {NAME OF FACILITY} Clinical Laboratory Bioterrorism Preparedness Plan: a Template

I. PURPOSE

A. The purpose of this protocol is to provide a formal description of how this laboratory will respond to a suspected or confirmed bioterrorism event. The laboratory may be called upon to assist in the diagnosis and management of patients who have been overtly or covertly exposed to a bioterrorism agent. The laboratory needs to promptly assist clinicians by providing them with accurate information on the selection, collection, and transport of specimens. In addition, the laboratory must handle these specimens in a manner that will result in the greatest probability of success in establishing a diagnosis and minimize the exposure of hospital personnel and patients to infectious agents.

B. In a suspected or confirmed bioterrorism event, immediate and effective communication with all appropriate institutional and medical personnel, and public health officials is imperative. If there is a specific, designated individual serving as the contact person for the institution's BT plan, this is the person who should be contacted.

C. An additional and very real possibility is that the laboratory will be the first to recognize that an organism isolated is a possible agent of bioterrorism.

II. LABORATORY BT CONTACT PROTOCOL: WHEN TO IMPLEMENT

A. If a possible BT agent is grown in the laboratory or detected by other laboratory means (as outlined in the laboratory protocols included in this document), place phone calls to the responsible physician and the following individuals noted below immediately. Contacting these individuals and the procedures required in the laboratory are NOT one-person tasks. Additional assistance from other technologists and laboratory support personnel is essential.

OR

B. If a specimen is submitted for detection of a BT agent as the result of a possible BT event, place phone calls to the individuals noted below immediately.

NOTE: Certain geographic areas are known to have natural human cases of infection due to BT agents (e.g., tularemia in Nantucket and Martha's Vineyard, Massachusetts, as well as Missouri, Oklahoma, and neighboring areas; and plague in much of the southwestern United States, especially New Mexico).

Microbiology Laboratory Supervisor: (xxx) xxx-xxxx

Microbiology Manager: (xxx) xxx-xxxx

Microbiology Laboratory Director: (xxx) xxx-xxxx

Infection Control Officer: (xxx) xxx-xxxx

Infectious Disease Physician: (xxx) xxx-xxxx

Local Health Department: (xxx) xxx-xxxx

State Health Department: (xxx) xxx-xxxx

Laboratory Director on Call (beeper no.): (xxx) xxx-xxxx

Clinical Pathologist on Call (beeper no.): (xxx) xxx-xxxx

Chief of Infectious Diseases: (xxx) xxx-xxxx

Chief of Pathology: (xxx) xxx-xxxx

Other: (xxx) xxx-xxxx

(Include contacts pertinent to your institution in a predetermined order, and delete those who are not to be contacted in your institution.)

III. THE LRN: LABORATORY RESPONSE NETWORK

The Laboratory Response Network (LRN) is a consortium and partnership of laboratories that provide immediate and sustained laboratory testing and communication in support of public health emergencies, particularly in response to acts of bioterrorism. The LRN is currently comprised of local, state, and federal public health laboratories in addition to private and commercial clinical laboratories, and selected food, water, agricultural, military, and veterinary testing laboratories. Other key federal partners include the Federal Bureau of Investigation (FBI), the Department of Defense (DOD), the Environmental Protection Agency (EPA), the Department of Agriculture (USDA), the Department of Justice (DOJ), the Department of Energy (DOE), the Food and Drug Administration (FDA), the Association of Public Health Laboratories (APHL), the National Institutes of Health (NIH), the American Association of Veterinary Laboratory Diagnosticians (AAVLD), and the American Society for Microbiology (ASM). All laboratories are regarded as partners and in some cases, registered members of the LRN. Preliminary testing and screening are performed primarily in a distributed rather than a centralized fashion to ensure a prompt and rapid initial response; a system of triage and referral of specimens ensures transfer of appropriate materials to specialty laboratories where sophisticated equipment, technologies, and expertise are applied to specimen analysis.

The goals of the LRN are to:

(1) Ensure that the nation's public health, clinical, and other select laboratories are prepared to detect and respond to a bioterrorism or chemical terrorism event in an appropriate and integrated manner.
(2) Ensure that all member reference laboratories collectively maintain state-of-the-art biodetection and diagnostic capabilities and surge capacity as well as secure

electronic communication of test results for the biological and chemical agents likely to be used in the commission of a biocrime or bioterrorism event.

(3) Work with other departments and agencies to ensure a successful federal response to an act of bioterrorism and to facilitate and optimize the ability of states to competently respond independently to biocrimes or public health emergencies in the state.
(4) Promote the CDC's and HHS' bioterrorism research agenda and CDC's internal response needs.
(5) Enlist an optimal number of registered participating LRN laboratories throughout the U.S. as determined by the LRN working group.

The LRN maintains the following:

(1) A registry and linkage of clinical and private laboratories in the U.S. that would include Sentinel and Reference laboratories.
(2) Complete, accurate, and standardized protocols for all levels of testing for agents deemed critical and likely to be used in the commission of biocrimes or acts of bioterrorism.
(3) Secure but accessible supply of standardized reagents and diagnostic technologies produced and maintained by the CDC.
(4) Secure electronic laboratory reporting that integrates with key epidemiologic, surveillance, and emergency response components
(5) Training and proficiency testing essential to the diagnostic process

Clinical laboratories play a critical role in the LRN. Their heightened awareness to the possibility of recovering the agents of bioterrorism from patient specimens and referral of suspect isolates to the appropriate public health reference laboratory is crucial (see *ASM's Laboratory Guideline on Packing and Shipping Diagnostic and Clinical Specimens, Infectious Substances, and Biological Agents, which can be downloaded from ASM's web site at* http://www.asm.org/images/pdf/Clinical/pack-ship-7-15-2011.pdf

Bioterrorism is defined as the "intentional use of microorganisms, or toxins, derived from living organisms, to produce disease and death in humans, animals, or plants." A bioterrorism event may be either overt or covert.

An **overt** attack would be accompanied by an announcement that a specific agent was released. These attacks elicit an immediate response by law enforcement and HAZMAT personnel. Public health officials will also be involved to assist in evaluating the risk and control of the disease. Samples (environmental, food, water, animals) for testing would be submitted directly to a public health reference laboratory, usually a state health laboratory.

A **covert** attack involves the release of an organism or toxin without an announcement. Days or weeks may pass before the release is noticed. The event would probably be signaled by a cluster of disease appearing after the incubation period. Emergency departments may be the first to observe unusual patterns of illness, while clinical laboratories would almost certainly detect the first cases of disease and raise suspicion of a possible event. Organisms isolated by the clinical

laboratory must be forwarded to the appropriate LRN reference laboratory, and public health officials are to be notified of the suspicious event that may be indicative of a bioterrorism incident. Public health officials in concert with law enforcement officials would determine if an attack has occurred, in addition to confirming the identification of the agent, and institute protective and preventive measures designed to minimize the spread of disease.

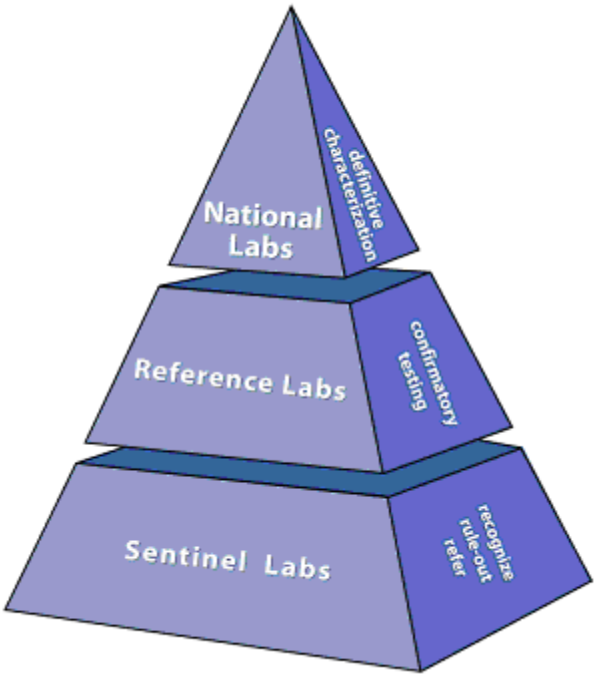
The LRN Structure: LRN Laboratories are designated as Sentinel, Reference, and National Laboratories (Fig. 1).

Sentinel Laboratories: Laboratories that are certified to perform high complexity testing under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) by the Centers for Medicare and Medicaid Services (CMS) for the applicable Microbiology specialty. Laboratory in-house testing includes Gram stains and at least one of the following: lower respiratory tract, wound or blood culture.

LRN Reference Laboratories: LRN reference laboratories are local and state public health laboratories, selected academic- or university-based laboratories, designated specialty laboratories (veterinary, water, food, chemical, military, agricultural) that possess the reagents and technology for definitive confirmation of organisms including toxin testing, referred by Sentinel laboratories. LRN Reference laboratories follow BSL-3 containment and practice guidelines. Contact your designated state public health laboratory for more information about the LRN reference laboratory located closest to you. A list of state public health laboratories can be downloaded at:
http://www.aphl.org/AboutAPHL/publications/Documents/PHPR_2012April_State-Public-Health-Laboratories-Emergency-Contact-Directory.pdf

LRN National Laboratories: LRN National Laboratories are Federal laboratories that have BSL-4 containment facilities and practice guidelines. The primary laboratory at this level is located at the CDC and specializes in the isolation and identification of BSL-4 agents such as Ebola, Marburg, and Smallpox virus. This laboratory also possesses the capability of advanced genetic characterization and archiving of all bioterrorism agents.

Fig. 1 The LRN Structure for Bioterrorism


National Labs
definitive characterization
Reference Labs
confirmatory testing
Sentinel Labs
recognize rule-out refer

IV. THE CLINICAL LABORATORY'S RESPONSIBILITY

As members of the LRN, Sentinel laboratories have access to the network and serve as "sentinels" for the early detection of and communication about a suspicious agent that cannot be ruled out as a possible bioterrorism-associated organism. Sentinel laboratories do not have access to the CDC secure website for Reference Laboratory Testing Protocols or reagents. Instead, Sentinel laboratories must utilize standardized testing protocols (ASM Sentinel Clinical Microbiology Laboratory Guidelines) that have been designed to utilize conventional tests to facilitate the "rule-out" or "referral" of a suspicious isolate to an LRN Reference laboratory.

The Sentinel laboratory is NOT responsible for and SHOULD NOT make the decision that a bioterrorism event has occurred; that responsibility rests with local, state, and federal health and law enforcement officials. **A designated individual within your facility (preferably the Infection Control Officer) should be notified of a suspicious agent, who in turns notifies the local public health officials. Under no circumstances should the laboratory contact law enforcement or public health officials. The exception is the need to contact the LRN Reference Laboratory for guidance in the disposition of the suspicious agent prior to referral for confirmatory testing.**

NOTE: In no case should the Sentinel laboratory accept environmental (powders, letters, packages), animal, food, or water specimens for examination, culture, or transport for bioterrorism-associated agents. Such specimens should be submitted directly to the nearest LRN Reference laboratory.

V. SENTINEL LEVEL CLINICAL MICROBIOLOGY LABORATORY GUIDELINES

The following Sentinel Level Clinical Microbiology Laboratory Guidelines can be downloaded at: http://www.asm.org/index.php/guidelines/sentinel-guidelines

Anthrax (*Bacillus anthracis*) – **revised** document posted

Botulism toxin – **revised** document posted

Brucellosis (*Brucella* species) – **revised** document posted

Plague (*Yersinia pestis*) – **revised** document posted

Tularemia (*Francisella tularensis*) – **revised** document posted

Q fever (*Coxiella burnetii*) – **revised** document posted

Burkholderia mallei and *B. pseudomallei* – **revised** document posted

Unidentified viruses – **revised** document posted

Staphylococcal enterotoxin B – **revised** document posted

VI. SHIPPING AND HANDLING OF INFECTIOUS MATERIALS

United States, international, and commercial regulations mandate the proper packing, documentation, and safe shipment of dangerous goods in order to protect the public, airline workers, couriers, and other persons who work for commercial shippers and who handle the dangerous goods during the many segments of the shipping process. In addition, proper packing and shipping of dangerous goods will reduce the exposure of the shipper to the risks of criminal and civil liabilities associated with shipping dangerous goods, particularly infectious substances.

The process of properly packing and shipping an infectious substance, a diagnostic specimen, or a biological agent is composed of the following sequential steps:

A. Training of all persons involved in the shipping process
B. Determination of the applicability of the regulations
C. Determination of any applicable shipping limitations
D. Classification of the substance to be shipped
E. Identification of the substance to be shipped
F. Selection of the appropriate packing instructions to use
G. Selection of appropriate packaging
H. Marking and labeling the package
I. Documentation of the shipment

Failure to follow governmental and commercial regulations for the packing and shipping of infectious substances and other dangerous goods can result in criminal prosecution and substantial financial penalties.

NOTICE: Regulations governing the transport of infectious substances change frequently. Shippers are responsible for being aware of these changes, being appropriately trained, certified, adhering to current regulations, interpreting applicable regulations for themselves and their facilities, and packing and shipping substances appropriately. Refer to IATA or USDOT regulations. ASM's Laboratory Guideline on Packing and Shipping Diagnostic and Clinical Specimens, Infectious Substances, and Biological Agents has been revised and is available at http://www.asm.org/images/pdf/Clinical/pack-ship-7-15-2011.pdf

VII. INFORMATION CHECKLIST

(This checklist may help in the gathering of information in a suspected bioterrorism event. The checklist is to be filled out by the shift operations manager, shift supervisor, or other designated personnel.)

Step	Task/data		Date/time completed	Signature
1. Name of patient(s), medical record number(s), patient location (s) and other pertinent information. A. B. C. D. E. F. G. H.				
2.	Who contacted lab about possibility of bioterrorism?			
3.	Person's (in step 2) phone number			
4.	Suspected bioterrorism agent(s) (e.g., anthrax, plague, etc., or unknown)	1. 2. 3. 4. 5.		

Step	Task/data		Date/time completed	Signature
5.	Contacted microbiology personnel	□ Yes □ No Who		
6.	Contacted clinical pathologist on call	□ Yes □ No		
7.	Contacted ID physician (**if instructed) and / or** IC practitioner **(if instructed)**	□ Yes □ No		
8.	If instructed to contact others *within* facility, write who and whether the person was available.	Who:		
		Contacted: □ Yes □ No		
		Who:		
		Contacted: □ Yes □ No		
9.	If instructed to contact others *outside* facility, write who and whether the person was available.	Who:		
		Contacted: □ Yes □ No		
		Who:		
		Contacted: □ Yes □ No		
10.	Specimens for suspected bioterrorism agents placed in the biological safety cabinet in the _____ part of the laboratory.	□ Yes □ No		

VIII. HANDLING OF POSSIBLE BT AGENT

NOTE: Under no circumstances are viral cultures to be set up if smallpox, Ebola virus, or another of the viral agents of bioterrorism is suspected.

A. A lead BT technologist should be appointed and be notified immediately that a suspected BT specimen or agent is in the laboratory. Laboratory workers are to be informed promptly of the name and medical record number of the person(s) with the suspected infection and, if appropriate, to treat other specimens from the patient(s) appropriately. This must be done in a manner that is in compliance with the Health Insurance Portability and Accountability Act (HIPAA).

B. All suspected BT specimens are to be processed in the biological safety cabinet located in **{fill in institution-specific information; whenever possible, this should be in a biological safety cabinet in a room that is under negative pressure}** while wearing appropriate personal protective equipment, such as gown, gloves, and mask.

C. Each of the plates, tubes, and blood culture bottles for which this applies must be labeled prominently: **"Possible highly infectious agent: [fill in name of agent]"**

D. Any growth from specimens is to be manipulated in the biological safety cabinet **{fill in institution-specific information}** while wearing appropriate personal protective equipment, such as gown, gloves, and mask.

E. As the culture is being worked up, the technologist(s) working on the culture(s) must be in close touch with the microbiology supervisor and medical director.

F. An identification of the organism is **NOT** the role of the Sentinel microbiology laboratory. An organism that is consistent with, for example, *Yersinia pestis*, will be forwarded to a LRN Reference or higher laboratory for definitive identification. **Do not perform any more manipulation of the cultures than is absolutely essential.**

IX. BT POLICY REVIEW DOCUMENTATION

Date reviewed	Employee signature

X. BT LABORATORY TRAINING DOCUMENTATION

Date	Employee signature	Signature of trainer

XI. THERAPY OF BT AGENTS

Specific treatment or prophylaxis for known or suspected exposure to bioterrorism agents will, in the setting of a bioterrorist event, likely be forthcoming from Public Health Authorities. In the absence of this information on the specific therapy for a given outbreak, a good source for information on treatment and prophylaxis is:

> Gilbert DN, Moellering RC, Sande MA. The Sanford Guide to Antimicrobial Therapy. 33rd ed. Table 1B: Prophylaxis and treatment of organisms of potential use as biological weapons, page 46. Antimicrobial Therapy, Inc., Hyde Park, Vermont.
>
> (http://www.sanfordguide.com/)

Please note that the table is “pathogen-based” and does not give information based upon a clinical syndrome in the absence of knowledge of the pathogen.

XII. APPENDIX

APPENDIX A: CDC Biosafety Level (BSL) Designations for Laboratories
Derived from reference BMBL: link = http://www.cdc.gov/biosafety/publications/bmbl5

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facility (secondary barriers)
1	Not known consistently to cause disease in healthy adults.	Standard microbiological procedures.	No Primary parries required. PPE: Laboratory coats and gloves, eye, face protection, as needed.	Laboratory bench and sink required.
2	Associated with human disease. Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure.	BSL-1 practice plus limited access, biohazard warning signs, sharps precautions, and a biosafety manual defining any needed waste decontamination or medical surveillance policies.	Primary barriers: Biosafety cabinet (BSC) or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials. PPE = Laboratory coats, gloves, and face and eye protection, as needed.	BSL-1 plus autoclave available.
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure.	BSL-2 practice plus controlled access, decontamination of all waste, decontamination of laboratory clothing before laundering.	Primary barriers: BSC or other physical containment devices used for all manipulations of agents. PPE = Protective laboratory clothing, gloves, and face. Eye and respiratory protection, as needed	BSL-2 plus physical separation from access corridors, self-closing double-door access, exhausted air not recirculated, negative airflow into the laboratory, hand washing sink near laboratory exit.
4	Dangerous/exotic agents which pose high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines of treatments. Agents with a close or identical antigenic relationship to an agent requiring BSL-4 until data are available to redesignate the level. Related agents with unknown risk of transmission.	BSL-3 practices plus clothing change before entering, shower on exit, all materials decontaminated on exit from facility.	Primary barriers: All procedures conducted in Class III BSC or Class I or II BSC in combination with full-body, air-supplied, positive-pressure personnel suit	BSL-3 plus separate building or isolated zone, dedicated supply/exhaust, vacuum and decontamination systems. Other requirements outlined in the text

APPENDIX B: Recommended BSL for BT agents

Agent	BSL		Specimen Exposure Risk	Recommended Laboratories Precautions	
	Specimen Handling	Culture Handling			
Alphaviruses	2	3	Blood, CSF. Tissue culture and animal inoculation studies should be performed at BSL-3 and are **NOT Sentinel laboratory procedures.**	BSL-2: Activities involving clinical material collection and transport	
Bacillus anthracis	2	3	Blood, skin lesion exudates, CSF, pleural fluid, sputum, and rarely urine and feces	BSL-2: Activities involving clinical material collection and diagnostic quantities of infectious cultures	BSL-3: Activities with high potential for aerosol or droplet production
Brucella spp.[a]	2	3	Blood, bone marrow, CSF, tissue, semen, and occasionally urine	BSL-2: Activities limited to collection, transport, and plating of clinical material	BSL-3: All activities involving manipulations of cultures
Burkholderia pseudomallei	2	3	Blood, sputum, CSF, tissue, abscesses, and urine	BSL-2: Activities limited to collection, transport, and plating of clinical material	BSL-3: All activities involving manipulations of cultures
Burkholderia mallei	2	3	Blood, sputum, CSF, tissue, abscesses, and urine	BSL-2: Activities limited to collection, transport, and plating of clinical material	BSL-3: All activities involving manipulations of cultures
Coxiella burnetii[b]	2	3	Blood, tissue, body fluids, feces. Manipulation of tissues from infected animals and tissue culture should be performed at BSL-3 and are **NOT Sentinel laboratory procedures**	BSL-2: Activities limited to collection and transport of clinical material, including serological examinations	
Clostridium botulinum[c]	2	3	Toxin may be present in food specimens, clinical material (serum, gastric, and feces). **TOXIN IS EXTREMELY POISONOUS!**	BSL-2: Activities with materials known to be or potentially containing toxin must be handled in a BSC (class II) with a lab coat, disposable surgical gloves, and a face shield (as needed).	BLS-3: Activities with high potential for aerosol or droplet production
Francisella tularensis[d]	2	3	Skin lesion exudates, respiratory secretions, CSF, blood, urine, tissues from infected animals, and fluids from infected arthropods	BLS-2: Activities limited to collection, transport, and plating of clinical material	BLS-3: All activities involving manipulations of cultures
Yersinia pestis[e]	2	3	Bubo fluid, blood, sputum, CSF, feces, and urine	BSL-2: Activities involving clinical material collection and diagnostic quantities of infectious cultures	BSL-3: Activities with high potential for aerosol or droplet production
Smallpox[f]	4	4	Lesion fluid or crusts, respiratory secretions, or tissue	BSL-2: Packing and shipping. Do **NOT** put in cell culture.	
Staphylococcal enterotoxin B	2	2	Toxin may be present in food specimens, clinical material (serum, gastric, urine, respiratory secretions, and feces), and isolates of *S. aureus.*	BSL-2: Activities involving clinical material collection and diagnostic quantities of infectious cultures	
VHF[g]	4	4	Blood, urine, respiratory, and throat secretions, semen, and tissue	BSL-2: Packing and shipping. Do **NOT** put in cell culture.	

[a]Laboratory-acquired brucellosis has occurred by “sniffing” cultures; aerosols generated by centrifugation; mouth pipetting; accidental parenteral inoculations; and sprays into eyes, nose, and mouth; by direct contact with clinical specimens; and when no breach in technique could be identified.

[b]Laboratory-acquired infections have been acquired from virulent phase I organisms due to infectious aerosols from cell culture and the use of embryonated eggs to propagate *C. burnetii.*

[c]Exposure to toxin is the primary laboratory hazard, since absorption can occur with direct contact with skin, eyes, or mucous membranes, including the respiratory tract. The toxin can be neutralized by 0.1 M sodium hydroxide. *C. botulinum* is inactivated by a 1:10 dilution of household bleach. Contact time is 20 min. If material contains both toxin and organisms, the spill must be sequentially treated with bleach and sodium hydroxide for a total contact time of 40 min.

[d]Laboratory-acquired tularemia infection has been more commonly associated with cultures than with clinical materials or animals. Direct skin/mucous membrane contact with cultures, parenteral inoculation, ingestion, and aerosol exposure have resulted in infection.

[e]Special care should be taken to avoid the generation of aerosols.

[f]Ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues are the primary hazards to laboratory workers.

[g]Respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and accidental parenteral inoculation are the primary hazards to laboratory workers.

APPENDIX C1
Specimen collection/handling for suspected Tier I BT agents

Disease/ Agent	Specimen Selection		Transport & Storage	Specimen Plating and Processing				
				SBA	CA	MAC	Stain	Other
Anthrax (*Bacillus anthracis*)	Possible *Bacillus anthracis* exposure in an asymptomatic patient	Swab of anterior nares: Only to be collected if so advised by local public health authorities	≤24 h, RT	No	No	No	None	Follow public health instructions on anterior nares swab ONLY if advised to collect these.
	Cutaneous	Vesicular stage: Collect fluid from intact vesicles on sterile swab(s). The organism is best demonstrated in this stage.	≤24 h, RT	X	X	X	Gram stain	
		Eschar stage: Without removing eschar, insert swab moistened in sterile saline beneath the edge of eschar, rotate, and collect lesion material.	≤24 h, RT	X	X	X	Gram stain	
		Vesicular stage and eschar stage: collect 2 punch biopsies Place one biopsy in 10% formalin to be sent to CDC for histopathology, immunohistochemical staining, and PCR.	One punch biopsy in 10% formalin. Once in formalin, can be stored until transported to CDC	No	No	No	Performed at CDC	Contact LRN Reference Level Laboratory before collecting specimen..
		Submit second biopsy for culture	≤24 h, RT	X	X	X	Gram stain	
		Blood cultures: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures.	Transport at RT. Incubate at 35-37°C per blood culture protocol	Blood culture bottles			Positive in some cases during late stages of disease	
		Purple-top tube (EDTA): for inpatients only, collect for direct Gram stain	≤2 h, RT	No	No	No	Gram stain	

Specimen collection/handling for suspected Tier I BT agents

Disease/ Agent	Specimen Selection		Transport & Storage	Specimen Plating and Processing				
				SBA	CA	MAC	Stain	Other
Anthrax (*Bacillus anthracis*) (continued)	Cutaneous (continued)	Red-top for serology; EDTA, heparin, and citrate are all acceptable for PCR	≤24 h, 4°C	No	No	No	No	Contact CDC for indication and direction for this testing.
	Gastro-intestinal	Stool: Collect 5-10 g in a clean, sterile, leakproof container.	≤24 h, 4°C	Inoculate routine stool plating media plus CNA or PEA.				Minimal recovery
		Blood cultures: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures.	Transport at RT. Incubate at 35-37°C per blood culture protocol.	Blood culture bottles			Positive in late stages of disease	
		Purple-top tube (EDTA): for inpatients only, collect for direct Gram stain	≤2 h, RT	No	No	No	Gram stain	
		Red-top tube for serology. EDTA, heparin, and citrate are all acceptable for PCR	≤24 h, 4°C	No	No	No	No	
	Inhalation	Sputum: Collect expectorated specimen into a sterile, leakproof container.	≤24 h, 4°C	X	X	X	Gram stain	Minimal recovery
		Pleural fluid: Collect specimen into sterile, leakproof container.	≤24 h, 4°C	X	X	X	Gram stain	Save excess (if any) for PCR.
		Blood cultures: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures.	Transport at RT. Incubate at 35-37°C per blood culture protocol.	Blood culture bottles			Positive in late stages of disease	
		Purple-top tube (EDTA): For inpatients only, collect for direct Gram stain.	≤2 h, RT	No	No	No	Gram stain	
		Red-top for serology; EDTA, heparin, and citrate are all acceptable for PCR	≤24 h, 4°C	No	No	No	No	
	Meningitis	Cerebrospinal fluid culture: Aseptically collect CSF per institutional procedure.	≤24 h, RT	X	X		Gram stain	May be seen in late stages of disease; consider adding broth medium such as brain heart infusion.

		Blood cultures: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures.	Transport at RT. Incubate at 35-37°C per blood culture protocol.	Blood culture bottles	Positive in late stages of disease

Specimen collection/handling for suspected Tier I BT agents

Disease/Agent	Specimen Selection					Transport & Storage	Specimen Handling
Botulism (botulinum toxin)	Specimen type	Clinical syndrome				Specimen(s) of choice for confirming botulism: 1. Serum 2. Wound/tissue 3. Stool 4. Incriminated food	
		Foodborne	Infant	Wound	Intention-al release (airborne)		
	Enema fluid – 20 ml	X	X		X	4°C	Contact LRN Reference level laboratory for instructions before collecting specimens.
	Food sample – 10-50g	X	X		X	4°C	Foods that support *C. botulinum* growth will have a pH of 3.5-7.0; most common pH is 5.5-6.5. Submit food in original container, placing individually in leakproof sealed transport devices.
	Gastric fluid – 20 ml	X,A				4°C	Collect up to 20 ml.
	Intestinal fluid – 20 ml	A	A			4°C	Autopsy: Intestinal contents from various areas of the small and large intestines should be provided.
	Nasal swab (anaerobic swab)				X	RT	For aerosolized botulinum toxin exposure, obtain nasal cultures for *C. botulinum* and serum for mouse toxicity testing.
	Serum – 15-20 mls	X,A		X	X	4°C	Serum should be obtained as soon as possible after the onset of symptoms and before antitoxin is given. Whole blood (30 ml [3 red-top or gold-top tubes]) is required for mouse toxicity testing. In infants, serum is generally not useful, since the toxin is quickly absorbed before serum can be obtained.

	Stool >25 g	X	X	X	X	4°C	Botulism has been confirmed in infants with only “pea-size” stools. Please note: Anticholinesterase given orally, as in patients with myasthenia gravis, has been shown to interfere with toxin testing.
	Vomitus - 20 ml	X				4°C	Collect up to 20 ml.
	Wound, tissue - anaerobic swab or transport system			`		Anaerobic swab or transport system Transport at RT	Exudate, tissue, or swabs must be collected and transported in an anaerobic transport system. Samples from an enema or feces should also be submitted, since the wound may not be the source of botulinum toxin.

Specimen collection/handling for suspected Tier I BT agents

Disease/Agent	Specimen Selection		Transport & Storage	Specimen Plating and Processing				
				SBA	CA	MAC	Stain	Other
Plague (*Yersinia pestis*)	Possible *Y. pestis* exposure in asymptomat-ic patient	No cultures or serology indicated						Follow public health instructions if advised to collect specimens.
	Bubonic	Blood cultures: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures.	Transport at RT. Incubate at 35-37°C per blood culture protocol.	Blood culture bottles			Gram stain of positive cultures	If suspicion of plague is high, obtain an additional set for incubation at RT (22-28°C) without shaking
		Red-top for serology; EDTA, heparin, and citrate are all acceptable for PCR	≤24 h, 4°C	No			No	Patients with negative cultures having a single titer, ≥1:10, specific to F1 antigen by agglutination would meet presumptive criteria.
		Lymph node (bubo) aspirate: Flushing with 1.0 ml of sterile saline may be needed to obtain material.	Transport at RT or 4°C if transport is delayed. Store at ≤24 h, 4°C.	X	X	X	Gram stain, Giemsa, Wright's stain	Contact LRN Reference lab before preparing smears for DFA.

		Tissue: Collect in sterile container with 1 to 2 drops of sterile, nonbacteriostatic saline.	Transport at RT or 4°C if transport is delayed. Store at ≤24 h, 4°C.	X	X	X	Gram stain, Giemsa, Wright's stain	Contact LRN Reference lab before preparing smears for DFA.

Specimen collection/handling for suspected Tier I BT agents

Disease/Agent	Specimen Selection		Transport & Storage	Specimen Plating and Processing				
				SBA	CA	MAC	Stain	Other
Plague (*Yersinia pestis*) (continued)	Bubonic (continued)	Throat: Collect routine throat culture using a swab collected into a sterile, leakproof container.	≤24 h, 4°C	X	X	X	Gram stain	Contact LRN Reference laboratory for additional guidance.
	Pneumonic	Sputum/throat: Collect routine throat culture using a swab or expectorated sputum collected into a sterile, leakproof container.	≤24 h, 4°C	X	X	X	Gram stain	Contact LRN Reference laboratory for additional guidance.
		Bronchial/tracheal wash: Collect per institution's procedure in an area dedicated to collecting respiratory specimens under isolation/containment circumstances, i.e., isolation chamber/"bubble."	≤24 h, 4°C	X	X	X	Gram stain	Contact LRN Reference laboratory for additional guidance.
		Blood cultures: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures.	Transport at RT. Incubate at 35-37°C per blood culture protocol.	Blood culture bottles			Gram stain of positive cultures	If suspicion of plague is high, obtain an additional set for incubation at RT (22-28°C) without shaking.
		Red-top for serology (acute and, if needed for diagnosis, convalescent serum in 14 days); EDTA, heparin, and citrate are all acceptable for PCR	≤24 h, 4°C	No			No	Patients with negative cultures having a single titer, ≥1:10, specific to F1 antigen by agglutination would meet presumptive criteria.

Specimen collection/handling for suspected Tier I BT agents

Disease/Agent	Specimen Selection		Transport & Storage	Specimen Plating and Processing				
				SBA	CA	MAC	Stain	Other
Plague (*Yersinia pestis*) (continued)	Meningitis	Blood cultures: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures.	Transport at RT. Incubate at 35-37°C per blood culture protocol.	Blood culture bottles			Gram stain of positive cultures	If suspicion of plague is high, obtain an additional set for incubation at RT (22-28°C) without shaking.
		Red-top for serology (acute and, if needed for diagnosis, convalescent serum in 14 days); EDTA, heparin, and citrate are all acceptable for PCR	≤24 h, 4°C	No			No	Patients with negative cultures having a single titer, ≥1:10, specific to F1 antigen by agglutination would meet presumptive criteria.
		Cerebrospinal fluid	Transport at RT. Store incubated at 35-37°C.	X	X		Gram stain	Can add broth culture at RT (22-28°C) without shaking.

Specimen collection/handling for suspected Tier I BT agents

Disease/Agent	Specimen Selection		Transport & Storage	Specimen Plating and Processing				
				SBA	CA	MAC	Stain	Other
Tularemia (*Francisella tularensis*)	Possible *Francisella tularensis* exposure in asymptomatic patient	No cultures or serology indicated						Follow public health instructions if advised to collect specimens.
	Oculo-glandular	Conjunctival scraping	≤24 h, 4°C	X	X	X	Gram stain	Add a BCYE plate and a plate selective for *Neisseria gonorrhoeae* such as modified Thayer-Martin. Manipulate cultures in a biological safety cabinet. Personal protective equipment includes gloves, gown, mask, and protective faceshield. All cultures should be taped shut during incubation. Contact LRN Reference laboratory for additional guidance.

Specimen collection/handling for suspected Tier I BT agents

Disease/Agent	Specimen Selection		Transport & Storage	Specimen Plating and Processing				
				SBA	CA	MAC	Stain	Other
Tularemia (*Francisella tularensis*) (continued)	Oculo-glandular (continued)	Lymph node aspirate: Flushing with 1.0 ml of sterile saline may be needed to obtain material.	Transport at RT, 4°C if transport is delayed. Store at ≤24 h, 4°C.	X	X	X	Gram stain	Add a BCYE plate and a plate selective for *Neisseria gonorrhoeae*, such as modified Thayer-Martin. Manipulate cultures in a biological safety cabinet. Personal protective equipment includes gloves, gown, mask, and protective faceshield. All cultures should be taped shut during incubation. Contact LRN Reference laboratory for additional guidance.
		Blood cultures: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures. Growth is more likely from aerobic bottle.	Transport at RT. Incubate at 35-37°C per blood culture protocol.	Blood culture bottles; subculture the broth to BCYE or chocolate plate and incubate aerobically.			Cultures should be manipulated in a biological safety cabinet. Personal protective equipment includes gloves, gown, mask, and protective faceshield. All cultures should be taped shut during incubation.	

Specimen collection/handling for suspected Tier I BT agents

Disease/Agent	Specimen Selection		Transport & Storage	Specimen Plating and Processing				
				SBA	CA	MAC	Stain	Other
Tularemia (*Francisella tularensis*) (continued)	Ulcero-glandular	Blood cultures: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures. Growth is more likely from aerobic bottle.	Transport at RT. Incubate at 35-37°C per blood culture protocol.	Blood culture bottles; subculture the broth to BCYE or chocolate plate and incubate aerobically.			Cultures should be manipulated in a biological safety cabinet. Personal protective equipment includes gloves, gown, mask, and protective faceshield. All cultures should be taped shut during incubation.	
		Ulcer or tissue: Collect biopsy (best specimen), scraping, or swab.	≤24 h, 4°C	X	X	X	Gram stain	Add a BCYE plate and a plate selective for *Neisseria gonorrhoeae* such as modified Thayer-Martin. Prepare smears for DFA referral if requested by state laboratory. Manipulate cultures in a biological safety cabinet. Personal protective equipment includes gloves, gown, mask, and protective faceshield. All cultures should be taped shut during incubation. Contact LRN Reference laboratory for additional guidance.
		Lymph node aspirate: Flushing with 1.0 ml of sterile saline may be needed to obtain material.	Transport at RT; 4°C if transport is delayed. Store at ≤24 h, 4°C.	X	X	X	Gram stain	
	Pneumonic	Sputum/throat: Collect routine throat culture using a swab or expectorated sputum collected into a sterile, leakproof container.	≤24 h, 4°C	X	X	X	Gram stain	
		Bronchial/tracheal wash: Collect per institution's procedure in an area dedicated to collecting respiratory specimens under isolation/containment circumstances, i.e., isolation chamber/"bubble."	≤24 h, 4°C	X	X	X	Gram stain	

Disease/Agent	Specimen Selection		Transport & Storage		Specimen Plating and Processing				
					SBA	CA	MAC	Stain	Other
Tularemia (*Francisella tularensis*) (continued)	Pneumonic (continued)	Blood cultures: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures. Growth is more likely from aerobic bottle.	Transport at RT. Incubate at 35-37°C per blood culture protocol.		Blood culture bottles; Subculture the broth to BCYE or chocolate plate and incubate aerobically.			Cultures should be manipulated in a biological safety cabinet. Personal protective equipment includes gloves, gown, mask, and protective faceshield. All cultures should be taped shut during incubation.	
		2 Red-tops tubes for serology (acute and, if needed for diagnosis, convalescent serum in 14 days). EDTA, heparin, and citrate are all acceptable for PCR.	≤2 h RT,	≤24 h, 4°C	No			Positive serology test would meet presumptive criteria. Confirmation requires culture identification or a 4-fold rise in titer.	

<table>
<tr><th rowspan="2">Disease/Agent</th><th colspan="2" rowspan="2">Specimen Selection</th><th rowspan="2">Transport & Storage</th><th colspan="6">Specimen Plating and Processing</th></tr>
<tr><th>SBA</th><th>CA</th><th>MAC</th><th>PC</th><th>Stain</th><th>Other</th></tr>
<tr><td rowspan="3">Melioidosis and glanders

(Burkholderia pseudomallei and Burkholderia mallei)</td><td>Possible Burkholderia pseudomallei or Burkholderia mallei exposure in asymptomatic patient</td><td>No cultures or serology indicated</td><td></td><td></td><td></td><td></td><td></td><td></td><td>Follow public health instructions if advised to collect specimens.</td></tr>
<tr><td rowspan="2">Clinical illness</td><td>Bone marrow</td><td>Transport within ≤2 h, at RT.
Store ≤24 h, at 4°C</td><td></td><td>X</td><td></td><td></td><td>Gram stain</td><td>B. pseudomallei is a small gram-negative bacillus that may demonstrate bipolar morphology on stain.

B. mallei is a small gram-negative coccobacillus.

Incubation should be at 35 to 37°C, ambient atmosphere; CO_2 incubation is acceptable.</td></tr>
<tr><td>Blood cultures: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures OR collect lysis-centrifugation (e.g., Isolator) blood cultures.</td><td>Transport at RT.
Incubate at 35-37°C per blood culture protocol.</td><td colspan="4">Blood culture bottles
OR
Collect lysis-centrifugation (e.g., Isolator) blood cultures and plate to:</td><td colspan="2" rowspan="2">Cultures should be manipulated in a biological safety cabinet. Personal protective equipment includes gloves, gown, mask, and protective faceshield. All cultures should be taped shut during incubation.
Incubation should be at 35 to 37°C, ambient atmosphere; CO_2 incubation is acceptable.</td></tr>
<tr><td></td><td>X</td><td></td><td></td></tr>
</table>

Specimen collection/handling for suspected Tier I BT agents

Disease/Agent	Specimen Selection		Transport & Storage	Specimen Plating and Processing					
				SBA	CA	MAC	PC	Stain	Other
Melioidosis and glanders (*Burkholderia pseudomallei* and *Burkholderia mallei*) (continued)	Clinical illness (continued)	Respiratory specimens, abscess material, wound specimens, urine	Transport within ≤2 h, at RT. Store ≤24 h, at 4°C.	X	X	X	X	Gram stain	If the laboratory has *B. cepacia* selective agar medium, it has been shown useful in isolation of *B. pseudomallei* for specimens in which indigenous microflora is likely to be encountered. Ashdown medium is a selective medium specifically designed for recovery of *B. pseudomallei*. This medium is not likely to be available in most Sentinel laboratories. Incubation should be at 35 to 37°C, ambient atmosphere; CO_2 incubation is acceptable.
		Red-top for serology (acute and, if needed for diagnosis, convalescent serum in 14 days); EDTA, heparin, and citrate are all acceptable for PCR	Transport within ~6 h, at 4°C. Store at -20°C to -70°C.	Contact the CDC for indications and directions for serologic diagnosis of *B. pseudomallei*					

[a]Abbreviations: A, autopsy; BCYE, buffered charcoal-yeast extract agar; C, centigrade; CA, chocolate agar; CNA, colistin-nalidixic acid agar; DFA, direct fluorescent antibody; MAC, MacConkey agar; PEA, phenylethyl alcohol blood agar; RT, room temperature; VHF, viral hemorrhagic fever; PC, selective medium for *Burkholderia cepacia*.

APPENDIX C2
Specimen collection/handling for suspected non-Tier I BT agents

Disease/ Agent	Specimen Selection		Transport & Storage	Specimen Plating and Processing				
				SBA	CA	MAC	Stain	Other
Brucellosis (*Brucella melitensis, B. abortus, B. suis, B. canis*)		Red-top for serology (acute and, if needed for diagnosis, convalescent serum in 21 days).	Transport in ≤2 h, at RT. Store at -20°C.	Specimen should be stored and shipped frozen at -20°C to LRN Reference Level laboratory.			Serologic diagnosis: 1. Single titer: ≥1:160 2. 4-fold rise 3. IgM NOTE: *B. canis* does not cross-react with standard serologic reagents.	
	Acute, subacute, or chronic	Blood: Collect 2 sets (1 set is 2 bottles) per institutional procedure for routine blood cultures.	Transport at RT. Incubate at 35-37°C	Non-automated blood culture bottles – incubate for 21 days, directly observe for turbidity daily and blind subculture every 7 days; terminal subculture of negative cultures. Automated blood culture systems – incubate for 10 days, perform terminal subcultures at 7 days.				Blood culture isolation rates vary from 15-70% depending on methods and length of incubation. Cultures should be manipulated in a biological safety cabinet. Personal protective equipment includes gloves, gown, mask, and protective face shield. All cultures should be taped shut during incubation.
		Bone marrow, spleen, or liver: Collect per institution's surgical/pathology procedure.	≤24 h, RT	X	X		Gram stain	Cultures should be manipulated in a biological safety cabinet. Personal protective equipment includes gloves, gown, mask, and protective faceshield. All cultures should be taped shut during incubation.
				Hold cultures for at least 7 days.				
	Meningitis	Cerebrospinal fluid culture: Aseptically collect CSF per institutional procedure.	≤24 h, RT	X	X		Gram stain	Cultures should be manipulated in a biological safety cabinet. Personal protective equipment includes gloves, gown, mask, and protective faceshield. All cultures should be taped shut during incubation. Consider adding broth medium such as brain heart infusion.
				Hold cultures for at least 7 days.				

Specimen collection/handling for suspected non-Tier I BT agents

Disease/Agent	Specimen Selection			Transport and Storage	Specimen Handling
	Specimen type	Foodborne	Airborne (intentional release)		
	Culture isolate	X	X	2-8ºC	Send *S. aureus* isolate for toxin testing on appropriate agar slant.
	Food specimen	X	X	2-8ºC	Food should be left in its original container if possible or placed in sterile unbreakable containers and labeled carefully. Place containers individually in leak proof containers (i.e., sealed plastic bags) to prevent cross-contamination during shipment. Empty containers with remnants of suspected contaminated foods can be examined.

Specimen collection/handling for suspected non-Tier I BT agents

<table>
<tr><th>Disease/agent</th><th colspan="3">Specimen Selection and Transport</th><th>Specimen Handling</th></tr>
<tr><td>Smallpox

(Variola virus)</td><td colspan="4">See CDC document “Specimen Collection and Transport Guidelines” for detailed instructions
http://www.bt.cdc.gov/agent/smallpox/response-plan/index.asp#guidec
(Click on Guide D)

NOTE: Only recently, successfully vaccinated personnel (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 suspected patients.</td></tr>
<tr><td rowspan="5"></td><td rowspan="3">Rash</td><td>Biopsy specimens</td><td rowspan="5">See CDC document “Specimen Collection and Transport Guidelines” for detailed instructions (Guide D).</td><td rowspan="5">1. A suspected case of smallpox should be reported <u>immediately</u> to the respective Local and State Health Departments for review.

2. And if, after review, smallpox is still suspected, further consultation with the CDC would be initiated by the state and/or local health department.

NOTE: Approval must be obtained prior to the shipment of potential smallpox patient clinical specimens to CDC.

3. At this time, review the packaging/shipping requirements with CDC and request assistance in coordinating a carrier for transport/shipment.
4. Hand carry all specimens and do not send specimens via pneumatic tube system.
5. Do not attempt viral cultures: this is a Biosafety Level 4 agent, and this could result in a very unsafe situation in which there is a significant amount of infectious virus.</td></tr>
<tr><td>Scabs</td></tr>
<tr><td>Vesicular fluid</td></tr>
<tr><td>Posterior tonsillar tissue swab</td><td>Swab</td></tr>
<tr><td>Blood</td><td>Use plastic tubes</td></tr>
<tr><td>Autopsy</td><td>Portions of skin containing lesions, liver, spleen, lung, lymph nodes, and/or kidney</td></tr>
</table>

<table>
<tr><th>Disease/agent</th><th>Specimen Selection</th><th>Transport & Storage</th><th>Specimen Handling</th></tr>
<tr><td rowspan="7">VHF

(Various viruses including Ebola, Marburg, Lassa, Machupo, Junin, Guanarito, Sabia, Crimean-Congo hemorrhagic fever, Rift Valley fever, Omsk hemorrhagic fever, Kyasanur Forest disease virus, and others)</td><td>Red-top for serology (acute and, if needed for diagnosis, convalescent serum in 14 days).</td><td>Transport within ~2 h, at RT.
Store at -20°C to -70°C.</td><td rowspan="5">Specific handling conditions are currently under development. Contact CDC to discuss proper collection and handling.

1. Double-bag each specimen.
2. Swab the exterior of the outside bag with disinfectant before removal from the patient’s room.
3. Do not use glass tubes.
4. Hand carry all specimens and do not send specimens via pneumatic tube system.

NOTE: Disposable equipment and sharps go into rigid containers containing disinfectant that are then autoclaved or incinerated. Double-bag refuse. The exterior of the outside bag is to be treated with disinfectant and then autoclaved or incinerated.

Do not attempt tissue culture isolation. This is only to be done in a Biosafety Level 4 facility.

In laboratory:
1. Strict barrier precautions are to be used. Personal protective equipment includes gloves, gown, mask, shoe covers, and protective faceshield.
2. Handle specimens in biological safety cabinet if possible.
3. Consider respiratory mask with HEPA filter.
4. Specimens should be centrifuged at low speed.</td></tr>
<tr><td>Viral culture, blood: Collect serum, heparinized plasma (green-top tube), or whole blood during acute febrile illness.</td><td>Transport at RT.
Store 4ºC or frozen on dry ice or liquid nitrogen.</td></tr>
<tr><td>Throat wash specimens: Mix with equal volume of viral transport medium.</td><td>Transport on wet ice.
Store at -40°C or colder.</td></tr>
<tr><td>Urine: Mix with equal volume of viral transport medium.</td><td>Transport on wet ice.
Store at -40°C or colder.</td></tr>
<tr><td>CSF, tissue, other specimens</td><td>As per discussion with CDC</td></tr>
<tr><td>Blood cultures: If clinical and travel history warrants, collect 2 sets (1 set is 2 bottles) of blood cultures per institutional procedure for routine blood cultures.</td><td>Transport at RT.
Incubate at 35-37°C per blood culture protocol.</td><td rowspan="2">Bacteremia with disseminated intravascular coagulation and malaria due to Plasmodium falciparum are two life-threatening and treatable clinical entities that can present with prominent clinical findings of hemorrhage and fever in a patient with a travel history to areas with VHF. Handle with precautions noted above. Continue to use the same precautions as above.</td></tr>
<tr><td>Malaria smear of peripheral blood: If clinical and travel history warrants</td><td>Lavender-top tube at RT</td></tr>
</table>

Disease/agent	Specimen Selection	Transport & Storage	Specimen Handling
Q. fever (*Coxiella burnetii*)	Serum: Collect 10 ml of serum (red-top, tiger-top, or gold-top tube) as soon as possible after onset of symptoms (acute) and with a follow-up specimen (convalescent) at ≥14 days for serological testing.	Transport within ~6 h, at 4°C. Store at -20°to -70	**Do not attempt tissue culture isolation**, as that could result in a very unsafe situation in which there is a significant amount of infectious organism. Sentinel laboratories should consult with State Public Health Laboratory Director (or designate) prior to or concurrent with testing if *C. burnetii* is suspected by the attending physician. Serology is available through commercial reference as well as public health laboratories.
	EDTA, heparin, and citrate are all acceptable for PCR	Transport within ~6 h, at 4°C. Store at 4°C.	
	Tissue, body fluids, others, including cell cultures and cell supernatants: Specimens can be kept at 2-8ºC if transported within 24 h. Store frozen at -70ºC or on dry ice.	Transport within <24 h, at 2-8°C. Store at -70°C or on dry ice.	

Specimen collection/handling for suspected non-Tier I BT agents

Disease/Agent	Specimen Selection	Transport & Storage	Specimen Handling
Alphaviruses (Includes Eastern equine, Western equine, Venezuelan equine encephalitis viruses and others)	Red-top for serology (acute and, if needed for diagnosis, convalescent serum in 14 days).	Transport within ~6 h, at 4°C. Store at -20°C to -70°C	**Do not attempt tissue culture isolation**, as that could result in a very unsafe situation in which there is a significant amount of infectious organism.
	EDTA, heparin, and citrate are all acceptable for PCR	Transport within ~6 h, at 4°C. Store at 4°C.	
	Cerebrospinal fluid: Specimens (greater than 1 ml) can be kept at 2-8ºC if transported within 24 h. If frozen, store at –70ºC and transport on dry ice.	Transport on wet ice. If already frozen, store at –70ºC and transport on dry ice.	
	Tissue, body fluids, others, including cell cultures and cell supernatants: Specimens can be kept at 2-8ºC if transported within 24 h. If frozen, store at -70ºC and transport on dry ice.	Transport on wet ice. If already frozen, store at –70ºC and transport on dry ice.	

[a]Abbreviations: A, autopsy; BCYE, buffered charcoal-yeast extract agar; C, centigrade; CA, chocolate agar; CNA, colistin-nalidixic acid agar; DFA, direct fluorescent antibody; MAC, MacConkey agar; PEA, phenylethyl alcohol blood agar; RT, room temperature; VHF, viral hemorrhagic fever; PC, selective medium for *Burkholderia cepacia*.

APPENDIX D: BT agent characteristics summary

Characteristic	*B. anthracis*	*Y. pestis*	*Burkholderia pseudomallei* and *B. mallei*	*F. tularensis*	*Brucella* spp.	Variola virus (smallpox)
Gram stain morphology	● Large gram-positive rod ● Nonmotile ● From blood agar: no capsule, central to subterminal spores that do not enlarge the cell ● From blood: capsule, no spores	● Plump gram-negative rod ● Gram stain: ± bipolar or "safety pin" appearance ● Wright-Giemsa: bipolar or "safety pin" appearance	● *B. pseudomallei*: small gram-negative rod ● *B. mallei*: small gram-negative coccobacillus ● Gram stain: ± bipolar or "safety pin" appearance (*B. pseudomallei*) ● Wright-Giemsa: bipolar or "safety pin" appearance (*B. pseudomallei*)	● Minute GNCB ● Poorly staining ● Smaller than *Haemophilus influenzae* ● Pleomorphic	● Tiny GNCB ● Faintly staining	
Growth	● Standard conditions ● Extremely rapid	● 28ºC optimal, without agitation ● 35-37ºC more slowly	● 35-37ºC ● Ambient atmosphere, though CO_2 is acceptable	● Aerobic conditions ● Growth is best on media containing cysteine, such as BCYE, but will often grow initially on chocolate or BA	● Grows in blood culture media ● Can require blind subculturing	● Grows in most cell lines ● Unusual or unrecognizable CPE
Colonial morphology (BA)	● Nonhemolytic ● Ground glass ● Irregular/wavy edges ● Tenacious ● "Beaten egg whites" when touched with loop	● Pinpoint at 24-48 h ● "Fried egg" or "hammered copper" or shiny at 48-72 h ● Nonhemolytic	*B. pseudomallei:* ● SBA: small, smooth creamy colonies in first 1 to 2 days, gradually changing after a few days to dry, wrinkled colonies similar to *Pseudomonas stutzeri* *B. mallei:* ● SBA: smooth, gray, translucent colonies without pigment	● Does not pass well on BA	● Small colonies ● Punctate after 48 h ● Nonhemolytic	
Tests	● Cat (+)	● Cat (+) ● Ox (–) ● Urease (–) ● MAC: Lac (–) ● Indole (–)	● Cat (+) ● Colistin (10 μg) and polymyxin B (300 U) (R) ● Motility (+) *B. pseudomallei* ● Motility (–) *B. mallei* ● Indole (–) ● Oxidase (+) *B. pseudomallei* ● Oxidase (+/-) *B. mallei* ● MAC: Lac (–) (B. *pseudomallei*) ● MAC: Lac (–) or NG (*B. mallei*)	● Cat wk (+) ● Ox (–) ● Urease (–) ● β-Lac (+) ● Satellite (–) ● MAC: NG	● Ox (+) ● Urease (+), though some are negative ● Satellite (–) ● MAC: Poor to NG	● CPE can be passed

APPENDIX E: BT agent clinical summary

Disease	Virulence factor(s)	Infective dose (ID)	Incubation period	Duration of illness	Person-to-person transmission[e]	Isolation precautions for hospitalized[f]	Persistence of organism
Inhalation anthrax	Exotoxin[a] capsule	Lower limit unknown, ID2 estimated at 9 spores[b]	1-6 days	3-5 days	No	Standard	>40 yr
Brucellosis	LPS;[c] PMN survival	10-100 organisms	5-60 days (usually 1-2 mo)	Weeks to months	Via breast milk[g] and sexually[h] (rare)	Standard	Water/soil, ~10 wk
Botulism	Neurotoxin	0.001 μg/kg is LD_{50} for type A	6 h to 10 days (usually 1-5 days)	Death in 24-72 h; lasts months if not lethal	No	Standard	Food/water, ~weeks
Glanders	Little studied, possible antiphagocytic capsule	Low	10-14 days via aerosol	Death in 7-10 days in septicemic form	YES (low)	Standard	Very stable
Melioidosis	Possibly LPS, exotoxin, intracellular survival, antiphagocytic capsule	Low	2 days to 26 yr	Days to months	YES (rare)[i]	Standard	Very stable in water/soil
Pneumonic plague	V and W antigens LPS (endotoxin) F1 antigen[d]	<100 organisms	2-3 days	1-6 days	YES (high)	Droplet[f]	Soil, up to 1 yr
Q fever	Intracellular survival LPS (endotoxin)	1-10 organisms	10–40 days	~2 wk (acute), months to years (chronic)	Rare[j]	Standard	Very stable
Smallpox		10-100 particles	7-17 days	~4 wk	YES (high)	Airborne[f]	Very stable
Staphylococcal enterotoxin B	Superantigen	0.0004 μg/kg incapacitation; LD_{50} is 0.02 μg/kg	3-12 h after inhalation	Hours	No	Standard	Resistant to freezing
Tularemia	Intracellular survival	10-50 organisms	2-10 days	≥2 wk	Single case report during autopsy	Standard	Moist soil, ~months
VHF	Varies with virus	1-10 particles	4-21 days	7-16 days	YES (moderate)	Airborne and contact[f]	Unstable

[a] *B. anthracis* exotoxin or exotoxins consist of three 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, which is thought to be the basis for Pasteur's attenuated anthrax vaccine used at Pouilly-le-Fort in 1881.

[b] The estimate that nine inhaled spores would infect 2% of the exposed human population is based on data from Science **266:**1202-1208, 1994. The dose needed to infect 50% of the exposed human population may be 8,000 or higher.

[c] 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.

[d]The V and W antigens and the F1 capsular antigens are only expressed at 7°C and not at the lower temperature of the flea (20 to 25°C).

[e]Periods of communicability are as follows: for **inhalation anthrax and botulism, none;** no evidence of person-to-person transmission; **pneumonic plague,** 72 h following initiation of appropriate antimicrobial therapy or until sputum culture is negative; **smallpox, approximately 3 weeks;** usually corresponds with the initial appearance of skin lesions to their final disappearance and is most infectious during the first week of rash via inhalation of virus released from oropharyngeal lesion secretions of the index case; **VHF, varies with virus, but at minimum, all for the duration of illness,** and for Ebola/Marburg transmission through semen may occur up to 7 weeks after clinical recovery.

[f]Guidelines for isolation precautions in hospitals can be found in Infect. Control Hosp. Epidemiol. **17:**53-80, 1996, in addition to the standard precautions that apply to all patients.

[g]Published reports of possible transmission of brucellosis via human breast milk may be found in Int. J. Infect. Dis. **4:**55-56, 2000; Ann. Trop. Paediatr. **10:**305-307, 1990; J. Infect. **26:**346-348, 1993; and Trop. Geogr .Med. **40:**151-152, 1988.

[h]Published reports of possible sexual transmission of brucellosis can be found in Lancet **i:**773, 1983; Aten Primaria **8:**165-166, 1991; Lancet **337:**848-849, 1991; Lancet **347:**1763, 1996; Lancet **337:**14-15, 1991; Infection **11:**313-314, 1983; and Lancet **348:**615, 1996.

[i]See Lancet **337:**1290-1291, 1991.

[j]Published reports of possible sexual transmission of Q fever can be found in Clin. Infect. Dis. **22:**1087-1088, 1996; and Clin. Infect. Dis. **33:**399-402, 2001.

APPENDIX F: Fever watch and post-exposure measures

Employee	Suspected Agent exposure	Base line serology collected	Base line serology tested/result	Fever medically monitored	Antibiotics given (dosage/route/ length)	Evaluation completed date

Fever watch and post exposure measures - Any employee that has been evaluated to have had a significant exposure to a possible select agent, they should be followed medically for any fever event and base line blood should be drawn for serology that targets the agent and/or antimcrobial prophylaxis should be given.

APPENDIX G: Alternative names for BT agents

Agent(s)	Other information that may appear on requisition
Bacillus anthracis	Anthrax, cutaneous anthrax, gastrointestinal anthrax, inhalation anthrax, anthrax meningitis, patient with hemorrhagic mediastinitis
Brucella melitensis, *B. suis*, *B. abortus*, *B. canis*	Brucellosis; history of ingestion of goat's milk; history of consumption of Mexican cheese; slaughterhouse worker; history of consumption of unpasteurized milk or cheese; contact with goats, sheep, cattle, or camels; laboratory worker with accident
Burkholderia mallei	*Pseudomonas mallei*, glanders, laboratory worker with accident
Burkholderia pseudomallei	*Pseudomonas pseudomallei*, melioidosis
Clostridium botulinum toxin	Botulism, botulinum toxin, botulism toxin, infant botulism, wound botulism, food from patient with botulism
Coxiella burnetii	Q fever, pneumonia and sheep exposure, pneumonia and goat exposure, culture-negative endocarditis
Crimean-Congo hemorrhagic fever virus	Congo-Crimean hemorrhagic fever virus, CCHF, viral hemorrhagic fever, VHF, hemorrhagic fever
Ebola virus	Ebola, viral hemorrhagic fever, VHF, hemorrhagic fever
Francisella tularensis	Tularemia, *Pasteurella tularensis*, rabbit fever, deerfly fever, history of skinning animals, history of rabbit contact, tularemic pneumonia, typhoidal tularemia, oculoglandular tularemia, ulceroglandular tularemia, glandular tularemia, pharyngeal tularemia
Guanarito virus	Venezuelan hemorrhagic fever virus, viral hemorrhagic fever, VHF, hemorrhagic fever
Hantaviruses (one causes a VHF)	Korean hemorrhagic fever, Sin Nombre virus, hantavirus pulmonary syndrome, viral hemorrhagic fever, VHF, hemorrhagic fever
Junin virus (a VHF)	Argentinian hemorrhagic fever virus, viral hemorrhagic fever, VHF, hemorrhagic fever
Lassa fever virus	Viral hemorrhagic fever, VHF, hemorrhagic fever
Machupo virus	Bolivian hemorrhagic fever virus, viral hemorrhagic fever, VHF, hemorrhagic fever
Marburg virus	Marburg, viral hemorrhagic fever, VHF, hemorrhagic fever
Nipah virus	Hendra-like virus, pig contact with encephalitis
Smallpox virus	Variola, smallpox
Staphylococcal enterotoxin B	*Staphylococcus aureus* enterotoxin B, *Staphylococcus aureus* enterotoxin, staphylococcal enterotoxin, food from patient with food poisoning
Viral hemorrhagic fever	Hemorrhagic fever, VHF
Yersinia pestis	Plague, bubonic plague, pneumonic plague, septicemic plague, bubo, *Pasteurella pestis*, plague meningitis

Appendix H: BT Readiness Checklist for Sentinel Laboratories

Does the laboratory have a biological safety cabinet?	YES ___ NO ___
Is the biological safety cabinet certified at least annually?	YES ___ NO ___
Does the laboratory have an autoclave?	YES ___ NO ___
If your laboratory does not have an autoclave, do you have appropriate procedure onsite destruction of suspect select agents?	YES ___ NO ___
Do you have a mechanism for tracking the destruction of suspect select agents?	YES ___ NO ___
Does the laboratory perform BSL2 or BSL3 practices?	YES ___ NO ___
Has someone in the laboratory completed LRN associated BT agent training?	YES ___ NO ___
Is someone in the laboratory certified in packaging and shipping of infectious substances within the last two years?	YES ___ NO ___
Does the laboratory participate in a BT readiness proficiency testing program offered by the state or CAP ?	YES ___ NO ___
Do you maintain reagents to perform rule-out testing for potential biological threat agents?	YES ___ NO ___
Does the laboratory have guidelines or protocols in place to handle clinical specimens suspected of containing a BT agent?	YES ___ NO ___
Do they include:	
Safe collection, processing and labeling of specimens?	YES ___ NO ___
Chain of custody?	YES ___ NO ___
Safe disposal/decontamination protocols?	YES ___ NO ___
Coordination with the institution's internal emergency management system?	YES ___ NO ___
Are there protocols to presumptively identify/rule out the following:	
Bacillus anthracis?	YES ___ NO ___
Brucella species?	YES ___ NO ___
Francisella tularensis?	YES ___ NO ___
Yersinia pestis?	YES ___ NO ___
Burkholderia mallei or B.pseudomallei?	YES ___ NO ___
Does the laboratory have a copy of the most recent ASM sentinel laboratory BT guidelines?	YES ___ NO ___

Does the laboratory staff know how and whom to contact at the LRN Reference laboratory regarding suspect BT agents?	YES ___ NO ___

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REFERENCES

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3. **Department of Health, Education, and Welfare.** 1974. Biohazards safety guide. Department of Health, Education, and Welfare, Bethesda, Md.
4. **Pike, R. M.** 1976. Laboratory-associated infections. Summary and analysis of 3921 cases. Health Lab. Sci. **13:**105-114.