University of Washington Medical Center
Clinical Microbiology Laboratory Document # 605.U.320.01

Bioterrorism Manual **Culture Processing of Bioterrorism Agents**		Effective: 2/12/18
Process Document	Written by: Karen LaFe	Reviewed by: Brett Norquist or Sarah Jensen Jen Vong
Revises or supersedes: Same of 3/2012, 3/2014, 12/2015, 2/2016		Revised by: Lynda Bui

ANNUAL REVIEW					
Reviewed by:	Date	Reviewed by:	Date	Reviewed by:	Date

I. Principle

In the event of a covert bioterrorist attack, the clinical microbiology laboratory may have the first indication that such an event has taken place. It is imperative that the employees of this laboratory are trained to recognize such events and to know what the procedures are. These events may be noticed first at the time of accessioning as different patients with similar symptoms have specimens sent for culture, or when initial gram stains are read, or at the bench level as cultures are being worked on and unusual isolates are recognized. **Any suspicions should be brought immediately to the attention of the lab manager and those individuals (BT Techs) designated to work with select agents.** In the event of either a covert or overt bioterrorism attack the clinical microbiology laboratory will work closely with federal and state officials to preserve the chain of evidence and to provide as much information as quickly as possible.

II. Specimens

A. Culture requests for patient specimens may appear as “Rule Out” or “R/O” of the organism name or the clinical disease name. It may also appear as: “R/O Select agent” or “Bioterrorism agent” or “BT agent”.

B. The following are bioterrorism select agents and their associated clinical disease names. These are agents that can be cultured in the laboratory.

Organism Name	**Clinical Disease Name**
Bacillus anthracis, *Bacillus cereus* biovar *anthracis*	Anthrax
Brucella abortus	Brucellosis
Brucella melitensis	Brucellosis
Brucella suis	Brucellosis
Burkholderia mallei	Glanders
Burkholderia pseudomallei	Melioidosis or Whitmore’s disease
Francisiella tularensis	Tularemia
Yersinia pestis	Plague

B. The following are bioterrorism select agents and their associated clinical disease names. These are agents that are **NOT CULTURED** in the laboratory.

Organism Name	Clinical Disease Name	Specific Instructions
Clostridium botulinum (neurotoxin producing species)	Botulism	Testing is performed by WSPHL. It must be approved prior to sending to the state lab.
Coxiella burnetii	Q Fever	This organism can only be cultured in cell lines but can be detected by molecular methods.

C. Specimens from a suspected bio-threat (BT) infected patient for culture in the laboratory may consist of any of the following.

Organism	Specimens
Bacillus anthracis *Bacillus cereus* biovar *anthracis*	Blood, CSF, pleural fluid, sputum, stool, rectal swab, vesicular fluid, eschar material, skin swab.
Brucella spp.	Blood, bone marrow, CSF, spleen, liver, joint fluid, abscesses, skin, stool.
Burkholderia mallei	Blood, bone marrow, sputum, bronchial washings, abscess material, wound swabs, urine.
Burkholderia pseudomallei	Blood, bone marrow, sputum, bronchial washings, abscess material, wound swabs, urine.
Francisella tularensis	Blood, sputum/throat, bronchial/tracheal washings, biopsied tissue, scraping of ulcer, ulcer swab, aspirate of tissue/lymph node, conjunctival scraping.
Yersinia pestis	Blood, bronchial wash (sputum/throat may be examined but not advised due to contamination with normal throat flora), CSF, tissue/bubo aspirate, tissue biopsy, skin scraping, lymph node.

D. Specimen accessioning: Log in all specimens as the appropriate routine culture for the type of specimen, but add in the specimen description "R/O <write name of agent>" as requested by physician.

III. Media and Supplies

A. Routine culture media

B. Household bleach

IV. Equipment

Standard microbiology equipment.

V. Quality Control

Media and stains are quality controlled per our standard protocols.

VI. Procedure

A. Notify the AD tech/manager of the presence of a "R/O select agent" specimen in the laboratory. In addition notify the laboratory with BIOALERT signage on the white board and on benches with

the patient information. As well as sending an alert via email uwmicro@uw.edu of the "R/O" request. The ADtech will send an appropriate alert to HMC if warranted.

B. Set up specimens in the biological safety cabinet (BSC).
1. Wear goggles, gloves and a protective gown.
2. If the culture request is for R/O *Bacillus anthracis* or *Bacillus cereus* biovar *anthracis*, prepare a 1:10 dilution of household bleach for soaking contaminated instruments (pipettes, needles, loops, glass slides, etc.) to kill possible *Bacillus* spores contained in the specimen. Soak instruments in the solution for 15 minutes before disposing in sharps container or other biohazard waste container.
3. Set up media according to the following charts.
4. Manually streak plates with disposable plastic loops. **Do not use the Isoplater or WASP**.
5. Place a "Suspect BT" sticker on each plate and on any broth.
6. Seal plates in re-sealable plastic bags.
7. Slides for Gram stain must be completely air-dried and heat-fixed before removal from the BSC.
8. Place all disposables, including gloves, in a double-bagged biohazard bag before removing them from the BSC.
10. If the culture is for R/O *B. anthracis* or *B. cereus* biovar *anthracis*, use additional 1:10 bleach solution to wipe down surfaces of the BSC. Then wipe again with regular disinfectant to remove bleach (to avoid the bleach's caustic effects).
11. Incubate all plates and broths in appropriate incubator and temperature.

C. Save all R/O Select agent specimens in double-biohazard bags and place in a container labeled "BT" in the specimen refrigerator

Organism	Specimens	Specimen Processing
R/O Anthrax or *Bacillus anthracis*, *B. cereus* biovar *anthracis*	Blood, CSF, pleural fluid, sputum, stool, rectal swab, vesicular fluid, eschar material, skin swab.	Routine methods are sufficient. For stool specimens, add CNA plate.
R/O Brucellosis or *Brucella* spp.	Blood, bone marrow, CSF, spleen, liver, joint fluid, abscesses, skin, stool.	Routine methods are sufficient. For blood cultures, incubate bottles for 10 days (log in as BLDEIC).
R/O Glanders or *Burkholderia mallei* R/O Melioidosis or Whitmore's disease or *Burkholderia pseudomallei*	Blood, bone marrow, sputum, bronchial washings, abscess material, wound swabs, urine.	Routine methods are sufficient.
R/O Tularemia or *Francisella tularensis*	Blood, sputum/throat, bronchial/tracheal washings, biopsied tissue, scraping of ulcer, ulcer swab, aspirate of tissue or lymph node, conjunctival scraping.	Routine methods are sufficient, but add CHOC if not part of usual set-up.
R/O Plague or *Yersinia pestis*	Blood, bronchial wash (sputum/throat may be examined but not advised due to contamination with normal throat flora), CSF, tissue/bubo aspirate, tissue biopsy, skin scraping, lymph node.	Routine methods are sufficient, but for non-blood specimens set up duplicate plates at 35°C and 30°C (in a bio-bag with a CO_2 generator).

VII. Safety

A. Risk to laboratory personnel from handling clinical specimens may be low, but it is important to minimize possible exposures to personnel as well as prevent contamination of the laboratory.

B. Standard laboratory practices are usually sufficient, but if a select agent is suspected, these precautions should be followed:

1. Wear googles, gloves and protective gowns when handling clinical specimens.
2. Wash immediately with soap and water if there is direct contact with a clinical specimen.
3. Avoid splashing or creating aerosols.
4. Perform culture set-up in the biosafety cabinet.
5. Blood cultures should be maintained in a closed system (blood culture bottles).
6. Keep culture plates covered at all times; minimize exposure when extracting specimens for testing.
7. Work on a smooth surface that can be cleaned easily and wipe with bleach or disinfectant regularly.
8. Utilize disposable sterile swabs and loops when culturing clinical specimens.

C. For accidental spills of material known or suspected to be contaminated with a select agent:

1. Flood with laboratory disinfectant solution or a 1:10 bleach solution, if *B. anthracis* is suspected.
2. Soak 5 minutes before cleaning up.

VIII. Limitations

Failure to isolate a BT agent from cultures does not totally rule out infection with that agent or other agents.

IX. References

A. **Baron, E.J**. 2001. Culture for Bioterrorism Agent. Stanford Hospital Clinical Laboratory Procedure. Standford, CA.

B. **CDC**. 2001. Anthrax Information for Laboratory Personnel. CDC Advisory 10-11-01.

WADOH. 2001. Culture of Bacterial Bioterrorism Agents. Washington State Department of Health publication.

C. **Craft, D.,** et. al. 2013. Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Francisella tularensis*. ASM, Washington, D.C.

E. **Gilligan, P.H**., et. al. 2013. Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species. ASM, Washington, D.C.

F. **Gilligan, P.H**., et. al. 2013. Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*. ASM, Washington, D.C.

G. **Shiflett, S.L.** et. al. 2013. Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: Botulinum toxin. ASM Washington. D.C

H. **Snyder, J.W**., et. al. 2017. Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Bacillus anthracis.* ASM, Washington, D.C.

I. **Sharp, S.E.**, et. Al. 2013. Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Yersinia pestisi.* ASM, Washington. D.C.

J. **CDC,** 2013. HHS and USDA Select Agents and Toxins. National Select Agent Registry. www.selectagents.gov

X. Revision Record

A. 3/2012
1. Section B.4. Removed statements that says lab does not identify or culture for *C. botulinum*.
2. Section VI.C. Added section on how to process R/O botulism cultures.
3. Section IX.A. Chart: Replaced "BacT/ALERT" with "blood culture instrument".

B. 3/2014
1. Removed all statements regarding testing for *Coccidioides immitis* or *C. posadasii*
2. Added: All specimens for rule out *Clostridium botulinum* or botulism will be sent to WSPHL. The lab will not identify or culture.
3. Removed: Processing instructions for *C. botulinum.*

C. 12/2015
1. Regional Center for Excellence Workgroup (research on *B. pseudomallei, F. tularensis,* and *Yersinia pestis)* no longer exists. All references removed from this document; revision to the procedure name performed.
2. Test codes for exposure cultures (BLDBT, FTULC, BPSEC, and YPSEC) removed from this procedure.

D. 2/2016
1. Section VI and VII. Removed requirement to perform processing of specimens in the Mycology biosafety cabinet.

E. 2/2018
1. Added *Bacillus cereus* biovar *anthracis* to any area with anthrax mentioned
2. Expanded bioalert notification process to laboratory
3. Converted from procedure to process document