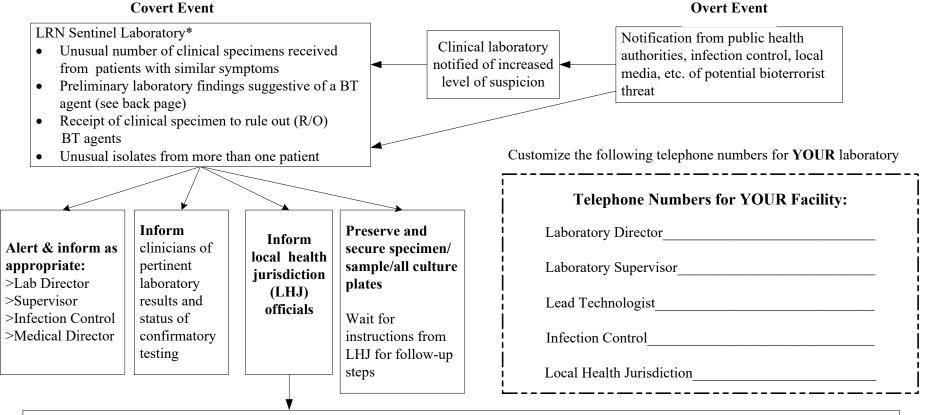
CLINICAL LABORATORY SUSPECTED BIOTERRORISM (BT) EVENT MANAGEMENT GUIDELINE

Washington State Clinical Laboratory Advisory Council

Originally published: April 2003

Reviewed/Revised: September 2005/April 2007/July 2009/October 2011/May 2014/Oct 2015/March 2018



Local Health Jurisdictions will:

- > Inform and involve Washington State Department of Health (DOH) Epidemiology staff and the DOH Public Health Laboratories to determine where suspect samples are to be sent for further analysis
- > Advise LRN Sentinel Laboratory on -which LRN Reference** laboratory to send the specimen/sample
 - -how to send the sample and special packing instructions
 - -include initial laboratory work-up

* LRN Sentinel Laboratory: Laboratories that perform blood and/or CSF cultures to RULE OUT a BT agent.

** LRN Reference Laboratory: Laboratories specifically authorized by the Centers for Disease Control and Prevention to perform testing to RULE IN the BT agent.

ENVIRONMENTAL SAMPLES: DO NOT ACCEPT any type of non-clinical specimen such as powders, other suspicious substances, or packages. Contact your local health jurisdiction. REFER all phone calls from people regarding environmental specimens to local law enforcement or to your local health jurisdiction.

Agent	Culture Methods	Recovery Time	Colony Morphology	Gram Stain Morphology	Presumptive ID	Action
Bacillus anthracis From: vesicle, sputum, CSF, stool, blood, tissue	BAP:35-37°C 5-10% CO ₂ CHOC:35-37°C 5-10% CO ₂ MAC: 35-37°C ambient air	8-24 hours	BAP/CHOC: Round w/regular edges, flat to slightly convex w/ground glass appearance, often w/comma shaped projections from colony edge which peaks when touched, non-hemolytic MAC: No growth	Large gram + rods often in short chains. Encapsulated w/sub- terminal spores (no swelling)	1)Ground glass appearance, no hemolysis on BAP; 2)No growth on MAC; 3)Large Gram + rods; 4) Catalase + 5)Non-motile	Refer to laboratory designated by the local health jurisdiction
<i>Francisella</i> <i>tularensis</i> From: blood, sputum, tissue, lymph node, or lesion aspirate	BAP: 35-37°C 5-10% CO ₂ CHOC: 35-37°C 5-10% CO ₂ MAC: 35-37°C ambient air Cystine supplemented agar (CHAB): 35-37°C 5-10% CO ₂ Thioglycollate broth: 35- 37°C 5-10% CO ₂	24-48 hrs, hold up to 10 days	BAP : Scant to no growth, may grow at first, but fails to grow with subsequent passage. CHOC/CHAB : Grey-white opaque usually too small to be seen at 24 hrs. At 48 hrs. usually 1-2 mm, white to grey to bluish grey, opaque, flat entire edge, smooth and shiny; MAC : No Growth	Tiny poorly counterstaining Gram - coccobacillus	1) Scant to no growth on BAP after 48 hrs. 1-2 mm grey white colonies on CHOC after 48 hrs. 2) No growth on MAC. 3) Tiny pleomorphic faintly staining Gram – coccobacillus. 4) Oxidase – 5) Catalase - or weakly + 6)Beta-lactamase + 7) no satellite growth.	Refer to Laboratory designated by the local health jurisdiction
Yersinia pestis From: blood, sputum, tissue, lymph node aspirate	BAP: 35-37°C 5-10% CO ₂ CHOC: 35-37°C 5-10% CO ₂ MAC: 35-37°C ambient air (Incubation at 25-28°C can aid in isolating <i>Y. pestis</i> from a contaminated sample.)	24-48 hrs, hold up to 7 days	BAP/CHOC : Grey-white, translucent colonies usually too small to see at 24 hrs. After 48 hrs, 1-2 mm, grey-white to slightly yellow and opaque. At 48-72 hrs, colonies have raised fried-egg or hammered copper appearance. Little to no hemolysis. MAC : Small non-lactose fermenting colonies.	Gram - rods mostly in single cells or pairs and in short chains in liquid media.	1)Pinpoint colonies at 24 hrs on SBA 2)Non-lactose fermenter may not be visible on MAC at 24 hrs 3)Gram – rods 4)Oxidase – 5)Indole – 6)Urease – 7) Catalase + 8) Motility – at 25°C	Refer to Laboratory designated by the local health jurisdiction
<i>Brucella sp.</i> From: blood, joint or abdominal fluid, tissue (spleen, liver abscess), bone marrow	BAP:35-37°C 5-10% CO ₂ CHOC:35-37°C 5-10% CO ₂ MAC: 35-37°C ambient air	24-48 hrs, hold up to 7 days	BAP/CHOC : Pinpoint colonies at 24 hrs; easily visible as white non-hemolytic colonies at 48 hrs. MAC : No growth.	Tiny gram – coccobacillus that stain faintly	1)Pinpoint colonies at 24 hrs easily visible at 48 hrs on BAP 2)No growth on MAC 3) Gram – coccobacillus that stain faintly 4) Oxidase + 5)Catalase + 6)Urease +	Refer to Laboratory designated by the local health jurisdiction
Burkholderia pseudomallei & mallei From: blood, sputum, tissue (biopsies, abscess aspirates), bone marrow, wound swabs, urine	BAP:35-37°C ambient/CO ₂ CHOC:35-37°C ambient/CO ₂ MAC: 35-37°C ambient/CO ₂	24 hrs, hold up to 5 days	 B. mallei BAP/CHOC: Smooth grey, translucent colonies in 48 hrs without pigment. MAC: Colonies may or may not grow. If present, they will be pinpoint at 48 hrs. B. pseudomallei BAP/CHOC: Small, smooth creamy colonies in 24-28 hrs. Colonies gradually change to dry wrinkled colonies. Nonhemolytic MAC: Good growth 	Gram – coccobacillus or small rods	1)BAP: poor growth at 24 hrs; better growth of grey, translucent colonies w/out pigment or hemolysis at 48 hrs. 2)Gram – coccobacillus or small rods 3)Indole – 4)Catalase +	Refer to Laboratory designated by the local health jurisdiction
Clostridium botulinum From: Feces, tissue, wound exudates, gastric contents, serum, food	Contact County Health Department. All testing will be conducted at the State Public Health Laboratory	Testing performed at the State Public Health Laboratory	Testing performed at the State Public Health Laboratory	Testing performed at the State Public Health Laboratory	Testing performed at the State Public Health Laboratory	Refer to Laboratory designated by the local health jurisdiction

LRN SENTINAL LABORATORY REFERENCE TABLE

References:

Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases 2013.; American Society for Microbiology website: http://www.asm.org/index.php/issues/sentinel-laboratory-guidelines