University of Washington Medical Center
Clinical Microbiology Laboratory Document # 605.U.103.06

Bioterrorism Manual **Procedure for Rule Out of *Bacillus anthracis***		Effective:
Written by: Karen LaFé	Reviewed by: Brett Norquist	Approved by: Brad Cookson
Supersedes: Procedure for Culture of Bioterrorism Agents: *Bacillus anthracis* of 11/03/2010, 3/31/14		Revised by: Lynda Bui

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

I. Principle

Bacillus anthracis, an obligate pathogen of animals and humans, is the causative agent of anthrax. Anthrax is primarily a disease of herbivores and can be transmitted to humans by direct contact with certain animal products, principally wool and hair. About 95% of human cases of anthrax are cutaneous infections caused by exposure to infected materials via breaks in the skin. Left untreated, the mortality rate for cutaneous anthrax is <20%. By contrast, mortality for inhalation anthrax, a severe hemorrhagic mediastinal adenitis resulting from inhalation of anthrax spores, is virtually 100% fatal. Anthrax is very rare in the United States. However, *B. anthracis* is always foremost on the list of potential agents used for biological warfare.

All specimens and cultures should be processed and examined with care in a biological safety cabinet. Every precaution should be taken to avoid the production of aerosols of the infected material. Once definitive identification of *B. anthracis* is known, the isolate is to be handled only by designated personnel.

II. Specimen Collection

Specimens that may be collected include material from cutaneous lesions, sputum, blood or any other site that may be infected.

III. Specimen Transport

Place specimen into a transenvelope with the requisition in the pocket. Transport to the laboratory without delay.

IV. Set-up Procedure

A. Refer to the "Procedure for Culture Processing of Bioterrorism Agents and Campus Health Requests for RCE Workgroup Exposure Specimens" in the Setup and Bioterrorism Manuals.

B. Incubation
   1. Temperature: 35-37°C.
   2. Atmosphere: ambient preferred, CO_2 is acceptable.
   3. Length of incubation: hold primary plates for at least 3 days; read daily. Examine plates within 18-24 hrs of incubation. Growth of *B. anthracis* may be observed as early as 8 hrs after incubation.

V. Identification

A. Stains and Smears: Gram Stain
   1. *B. anthracis* is a large gram-positive rod (1-1.5 X 3-5 µm).
   2. Vegetative cells seen on Gram-stained smears of clinical specimens often occur in short

chains of two to four cells that are encapsulated. Endospores are not commonly seen in direct smears of clinical specimens.

3. Gram stains from colonies grown on BAP appear as long chains of nonencapsulated Gram-positive bacilli. If present, the spores are oval and located centrally or subterminally and do not cause swelling of the vegetative cell.

B. Colony characteristics of *B. anthracis*

B. anthracis grows well on BAP and CHOC, but not on MAC. Colonies are round with irregular edges, flat or slightly convex with a ground glass appearance. There are often "comma-shaped" projections from the edge of the colony, producing the "Medusa head" shape. The colonies are nonhemolytic on BAP and have a tenacious consistency that when teased with a loop, the growth will stand up like beaten egg whites.

C. Testing and Observation in the BSC

1. Hemolysis: Nonhemolytic on BA
2. Growth on MAC: no growth
2. Catalase: positive
3. Motility: negative
   a. Using a sterile stick, remove a portion of growth from an isolated, suspect colony after 18-24 hrs incubation.
   b. Inoculate the motility medium by carefully stabbing the stick 3-4 cm into the medium and then drawing the stick directly back out so that a single line of inoculum can be observed.
   c. Incubate the tube at 35°C in ambient atmosphere for 18-24 hrs.
4. *Bacillus anthracis* is ruled out if the catalase test is negative or the motility test is positive or the isolate is beta hemolytic.

VI. Interpretation and Reporting

A. Any isolate that cannot be ruled out as *B. anthracis* should be brought immediately to the attention of the lab manager and BT tech/lead).

B. If it has been determined that the isolate must be sent for identification, report as "*Bacillus* species: Identification to follow. Sent to State Lab for testing (STSL)" until a result is obtained from WSPHL.

C. **Follow the notification instructions and regulatory guidelines outlined in the procedure "Select Agent Reporting and Bioterrorism Preparedness".**

D. **Secure all culture media and specimen(s). Hand over to the BT tech.**

IX. Antimicrobial Susceptibility Testing

A. Susceptibility testing is not recommended and is not done in our laboratory.

B. If the physician requests susceptibility testing, it will be performed at WSPHL.

C. The treatment of choice is penicillin.

D. Other antibiotics that have proven effective include gentamicin, erythromycin, and chloramphenicol. Ciprofloxacin and doxycycline may also be efficacious.

X. BT Agent Clinical Summary

Source: "Sentinel Level Clinical Laboratory Guidelines: Clinical Laboratory Bioterrorism

Readiness Plan, Appendix E"

A. Virulence Factor: Exotoxin capsule
B. Infective Dose: lower limit unknown, estimated at 9 spores
C. Incubation period: 1-6 days
D. Duration of illness: 3-5 days
E. Person to person transmission: No
F. In clinical specimens *B. anthracis* cells are primarily vegetative and not easily transmitted.
G. Primary hazards to laboratory personnel are direct and indirect contact of intact and broken skin with cultures and accidental parenteral inoculation.

XI. Quality Control

Refer to individual culture procedures in the Bacteriology Manual.

XII. References

A. **Brachman, P.S. and A.M. Friedlander.** Anthrax, p. 729-739. In S.A. Plotkin and E.A. Mortimer, Jr. (ed), Vaccines. W.B. Saunders, Philadelphia, PA.

B. **Cieslak, T.J. and E.M. Eitzen, Jr.** 1999. Clinical and epidemiologic principles of anthrax. Emerg. Infect. Dis. 5:552-555.

C. **Dutz, W. and E. Kohout**. 1971. Anthrax. Pathol. Annu. 6:209-248.

D. **Gilchrist, M.J.R., W.P. McKinney, J.M. Miller, and A.S. Weissfeld.** 2000. Cumitech 33, Laboratory Safety, management, and diagnosis of biological agents associated with bioterrorism. Coordinating ed., J.W. Snyder. ASM Press, Washington. D.C.

E. **Koneman, E. et al.** 1997. Color Atlas and Textbook of Diagnostic Microbiology. Lippincott, Philadelphia and New York.

F. **Lew, D.P.** 2000. *Bacillus anthracis* (Anthrax). P 2215-2220. In G.L. Mandell, J.E. Bennett, and R. Dolin (ed), Principles and Pracitice of Infectious Disease, 5th ed. Churchill Livingston, Philadelphia, PA.

G. **Logan, N.A. and P.C. Turnbull.** 1999. *Bacillus* and recently derived genera, p. 357-369. In P.R. Murray, E. J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed) Manual of Clinical Microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

H. **Murray, P. et al.** 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

I. **Weissfeld, A. and Snyder J.W.** 2013 Sentinel Level Clinical Laboratory Guidelines: *Bacillus anthracis*

XIII. Attachments

A. Guidelines for GPR and GNR BT flowcharts

XIV. Revision Record

A. 11/3/2010: Revisions made under Section VIII regarding calling WSPHL for an isolate that cannot be ruled out as *B. anthracis*.
B. 11/3/2010: Section IX expanded regarding susceptibility testing not performed in our laboratory.
C. 3/2014: Added Section X, BT Agent Clinical Summary
D. 3/2015: Reorganized for flow; Section V. C. removed 25°C motility; replaced *Bacillus* flowchart

University of Washington Medical Center
Clinical Microbiology Laboratory Document # 605.U.104.05

Bioterrorism Manual **Procedure for Rule Out of *Brucella* spp.**		Effective:
Written by: Karen LaFé	Reviewed by: Sarah Jensen	Approved by: Brad Cookson
Revises or supersedes: Procedure for Culture of Bioterrorism Agents: Brucella spp. of 10/20/2009, 3/31/14		Revised by: Lynda Bui

ANNUAL REVIEW			
Reviewed by:	Date	Reviewed by:	Date

I. Principle

Brucellosis is a zoonotic disease and domestic animals serve as a reservoir. The members of the genus *Brucella* are important veterinary pathogens. Examples include *B. abortus* which most commonly infect cattle, *B. melitensis* which naturally infects goats and sheep, and *B. suis* which most commonly infects swine. Transmission of brucellosis can be the result of ingestion, direct contact via skin abrasions and mucous membranes and inhalation. Risk factors for infection include the handling of infected animals, ingestion of contaminated animal products such as unpasteurized milk or cheese and the handling of cultures of *Brucella* spp. in laboratories.

Brucellois may present as a systemic illness (fever, chill, weakness, malaise, etc.) and/or may include involvement of the liver, bones and joints, CSF, skin, etc. The diagnosis of brucellosis is definitively established by culture of the organism. The clinician should alert the laboratory so cultures can be held longer which is sometimes necessary for isolation of *Brucella* spp.

All specimens and cultures should be processed and examined with care in a biological safety cabinet. Every precaution should be taken to avoid the production of aerosols of the infected material. Once definitive identification of *Brucella abortus, B. melitensis,* or *B. suis* is known, the isolate is to be handled only by designated personnel.

II. Specimen Collection

Blood and bone marrow aspirates are the clinical specimens from which *Brucella* is most commonly isolated. Other clinical sites from which *Brucella* spp. have been recovered are lymph nodes, synovial fluid, CSF, abscess aspirates, and liver and spleen biopsies.

III. Specimen Transport

Transport specimens in a trans-envelope with requisition. Specimens should be received as soon as possible after collection for optimal recovery.

IV. Set-up Procedure

Refer to the "Procedure for Culture Processing of Bioterrorism Agents and Campus Health Requests for RCE Workgroup Exposure Specimens" in the Setup and Bioterrorism Manuals.

All patient specimens should be handled while wearing gloves and gowns and working in a biosafety cabinet. Subcultures should be performed in a BSC and incubated in 5-10% CO_2. Enclose plates in resealable plastic bags and all further testing should be performed only in the BSC.

V. Cultures

A. Inoculation and plating procedure: standard media should be used according to normal laboratory procedures for the type of specimen. Refer to the "Procedure for Culture Processing of Bioterrorism Agents and Campus Health Requests for RCE Workgroup Exposure Specimens" in the Setup and Bioterrorism Manuals.

B. Incubation
   1. Temperature: 35-37°C.
   2. Atmosphere: 5-10% CO_2.
   3. Length of incubation
      a. Blood cultures and fluid cultures in blood culture bottles: incubate for 10 days. Perform terminal Acridine orange stain and subculture to CHOC and BRU at day 10. Hold subcultures 4 days.
      b. Tissues: Incubate plates for 3 days, broths for 7 days.

VI. Identification

A. Gram stain
   1. *Brucella* are small (0.4 X 0.8 µm), gram-negative coccobacilli that can be visualized directly from positive blood culture bottles or Gram stains of colonies from primary media. They stain poorly and do not cluster.
   2. Refer to the ASM images for examples of *Brucella* gram stain.

B. Colony characteristics
   1. *Brucella* species will grow on both BAP and CHOC but not on MAC.
   2. Colonies are pinpoint at 24hrs and show "dust-like" growth after overnight incubation. They are easily visible as white, non-hemolytic, non-mucoid colonies at 48hrs. Pinpoint colonies have infrequently been observed on MAC after 7days.
   3. Refer to the ASM images for examples of *Brucella* growth.

C. **WARNING**
   1. ***Brucella* has been responsible for many laboratory-acquired infections.**
   2. The identification of *Brucella* species should not be attempted with commercial identification systems such as the Vitek.
   3. The Bruker mass spectrometry library DOES NOT contain bioterrorism agents. Results may be reported as "no reliable information".
   4. Other organisms that can be confused with *Brucella* species because they are urease positive are *Oligella ureolytica* (usually found only in urine), *Psychrobacter phenylpyruvicus, Psychrobacter immobilis,* and *Bordetella bronchiseptica* (motile).

D. Testing and Observation in the BSC
   1. Gram stain: Small, gram-negative coccobacilli.
   2. Grows only in aerobic blood culture after 2-4 days.
   3. Grows as typical colonies on BA and CHOC within 48 hrs.
   4. Positive for oxidase and catalase
   5. Positive for urea
      a. Some *Brucella* strains have a delayed (72 hours) urease test and may give a negative reaction. Mass spec may be performed **at 48hrs** if all other tests rule out a possible *Brucella*. Consult with lead before proceeding with mass spec identification. (Perform extraction in BSC.)
   5. *Brucella* spp. are ruled out if the *S. aureus* satellite test is positive.

VII. Interpretation and Reporting

A. Any isolate where *Brucella* spp. cannot be ruled out should be brought immediately to the attention of the director, lab manager, and BT Techs/lead.

B. If it has been determined that the isolate must be sent for identification, report "X+ Gram negative rods: Identification to Follow. Sent to State Lab for testing (STSL)"

C. **Follow the notification instructions and regulatory guidelines outlined in the procedure "Select Agent Reporting and Bioterrorism Preparedness".**

D. If the organism is determined to be a select agent (either by the State Lab or the molecular section), refer to the above procedure for reporting.

E. **Secure all culture media and specimen(s). Hand over to the BT tech.**

VIII. Limitations

A. *B. abortus*, *B. melitensis*, and *B. suis* are all oxidase-positive organism. *B. canis* isolates may be oxidase-variable.

B. Using the Christensen's tube test, urea hydrolysis can be observed in as early as 15 min incubation with *B. suis* strains and within 1 day of incubation with most strains of *B. abortus*, and *B. melitensis*. Some *B. melitensis* strains take even longer to be positive.

C. Because there are a number of urea-positive, fastidious tiny gram-negative rods, the definitive identification of *Brucella* is performed by DNA sequencing. However, isolation of an organism with the characteristics of *Brucella* listed in this procedure from a blood or normally sterile site is most likely *Brucella*.

IX. Antimicrobial Susceptibility Testing

A. Antimicrobial susceptibility testing of *Brucella* is not performed by this laboratory.

B. Refer to the WSPHL for testing is a physician requests it.

X. BT Agent Clinical Summary

Source: "Sentinel Level Clinical Laboratory Guidelines: Clinical Laboratory Bioterrorism Readiness Plan, Appendix E"

A. Virulence factor: Lipopolysaccharide; PMN survival
B. Infective dose: 10-100 organisms (most commonly reported laboratory acquired infection)
C. Incubation period: 5-60 days (usually 1-2 months)
D. Duration of illness: Weeks to months
E. Person to person transmission: Via breast milk and sexually (rare)
F. Laboratory hazards include direct contact of skin or mucous membranes, accidental parenteral inoculation or ingestion, and exposure to aerosols generated during manipulation of cultures.

XI. Quality Control

Refer to individual culture procedures in the Bacteriology Manual.

XII. References

A. **Isenberg, H.** et al. 2004. Clinical Microbiology Procedures Handbook. ASM Press, Washington, D.C.

B. **Murray, P**. et al. 2003. Manual of Clinical Microbiology 8th ed. ASM Press, Washington, D.C.

C. **Gilligan, P.H. and York, M.K.** 2013. Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism, *Brucella* species. ASM Press, Washington. D.C.

XIII. Attachments

A. Guidelines for GPR and GNR BT flowcharts

B. Gram Negative Rod Select Agent Identification Chart

XIV. Revision Record

A. 3/2014: Added section BT Agent Clinical Summary

B. 3/2015:
1. Reorganization for flow; discontinued attachment "Rule Out *Brucella* spp. BT Algorithm #2: Gram Negative Bacilli or Coccobacilli" and added new consolidate chart
2. Section VI. Added statement regarding mass spec library does not contain BT agents

University of Washington Medical Center
Clinical Microbiology Laboratory Document # 605.U.105.05

Bioterrorism Manual **Procedure for Rule Out of *Burkholderia mallei* & *B. pseudomallei***		Effective:
Written by: Karen LaFé	Reviewed by: Sarah Jensen	Approved by: Brad Cookson
Revises or supersedes: Same of 10/20/2009, 5/2014		Revised by: L. Bui

ANNUAL REVIEW			
Reviewed by:	Date	Reviewed by:	Date

I. Principle

Burkholderia mallei is the causative agent of glanders. This disease primarily affects horses, donkeys, and mules. Human infection is rare and is usually a result of prolonged contact with an infected animal. The most recent human cases of this disease have occurred in laboratory workers since it takes very few organisms to cause infection. It is considered a potential agent of bioterrorism.

Burkholderia pseudomallei is an environmental organism found in soil and water and is most likely obtained naturally by direct contact with, or aerosols from, environmental sources. It is the causative agent of melioidosis or Whitmore's disease. Melioidosis is similar to glanders. Person to person transmission has been reported but this is extremely rare. *B. pseudomallei* is an organism that has been considered a potential agent for bioterrorism.

All specimens and cultures should be processed and examined with care in a biological safety cabinet. Every precaution should be taken to avoid the production of aerosols of the infected material. Once definitive identification of *B. mallei* or *B. pseudomallei* is known, the isolate is to be handled only by designated personnel.

II. Specimens

Blood, sputum or bronchoscopically obtained specimens, abscess material, wound swab and urine are acceptable specimens.

III. Specimen Transport

Transport to the laboratory without delay.

IV. Set-up Procedure

A. Refer to the "Procedure for Culture Processing of Bioterrorism Agents and Campus Health Requests for RCE Workgroup Exposure Specimens" in the Setup and Bioterrorism Manuals.

B. Culture Incubation
1. Temperature: 35-37°C.
2. Atmosphere: Ambient; CO_2 acceptable.
3. Length of incubation: Hold primary plates for a minimum of 5 days; read daily. *B. pseudomallei* will reliably grow with 5 days of incubation from blood cultures. *B. mallei* will not grow as rapidly as *B. pseudomallei* and may require extended incubation.

V. Gram Stain, Colony, and Biochemical Characteristics

A. **All cultures suspected on containing *B. mallei* or *B. pseudomallei* should be handled in a biological safety cabinet.**

B. **Use disposable sterile loops and sticks as needed for testing. Tape plate shut or enclose in plastic baggies to prevent inadvertent opening.**

C. *B. mallei* or *B. pseudomallei* is suspected of and cannot be ruled out if the organism fulfills the following characteristics:

Characteristic/ Biochemical Test	***B. mallei***	***B. pseudomallei***
Gram stain	Small, straight or slightly curved gram negative coccobacillus (1.5-3µm length x 0.5-1µm diameter)	Small, straight or slightly curved gram negative rod; may have bipolar staining in direct specimens (2-5µm length x 0.4-0.8µm diameter)
Colony growth BA	Gray, translucent colonies, nonhemolytic, nonpigmented at 48hrs.	White colonies, nonhemolytic, nonpigmented at 48hrs; may be wrinkled (like P. stutzeri) Notes: May have distinct musty odor which can be smelled without opening the lid
Colony growth MAC	No growth to pinpoint colonies at 48hrs	Growth; colonies appear pink but is not fermentation
Oxidase	Variable (most negative)	Positive
Catalase	Positive	Positive
Spot indole	Negative	Negative
Arginine	Positive	Positive
Motility agar	Non-motile	Motile
Growth 42°C Compare by setting up at 35°C and 42°C.	No growth	Growth
Colistin (10µg)* Interpretation: Any zone is susceptible	No zone	No zone
Amoxicillin-Clavulanic acid (20/10µg)* Interpretation: ≥18mm=susceptible	Susceptible	Susceptible (Rare resistance)
Penicillin (10U)* Interpretation: ≤28mm = resistant ≥29mm=susceptible	Resistant	Resistant

***Perform using the Kirby-Bauer disk diffusion method (0.5 McFarland). If it does not grow on MH, BAP or CHOC can be used solely for identification.**

D. **WARNING**

1. Commercial identification systems do not reliably identify these two organisms. Do not use.
2. *B. pseudomallei* cannot be differentiated from *B. thailandensis* or *B. oklahomensis* using the biochemical tests described above. The Bruker mass spectrometry library does not contain bioterrorism agents.
3. Organisms that can be misidentifications:
   a. *Burkholderia cepacia* complex (question ID if non-cystic fibrosis patient)
   b. *Chromobacter violaceum*

VI. Interpretation and Reporting

A. Any isolate that cannot be ruled out as *B. mallei or B. pseudomallei* should be brought to the attention of the lab manager and BT tech/lead.

B. Definitive identification will be performed by the Washington State Public Health Laboratory (WSPHL).

C. **Follow the notification instructions and regulatory guidelines outlined in the procedure "Select Agent Reporting and Bioterrorism Preparedness"**.

D. In the LIS, report as:

Organism to Rule Out	Preliminary report
B. mallei	Gram negative rod: Identification to follow. Sent to State Lab for testing (GNR-C-IDTF-STSL)
B. pseudomallei	Non lactose fermenting gram negative rod: Identification to follow. Sent to State Lab for Testing (NLF-C-IDTF-STSL)

VII. Antimicrobial Susceptibility Testing

A. Antimicrobial susceptibility testing is not performed in this laboratory.

B. *Burkholderia mallei*: There are no specific interpretive criteria for susceptibility testing for this organism. It is likely that treatment strategies used for *B. pseudomallei* will be effective against *B. mallei*.

C. *Burkholderia pseudomallei*: Current recommendations are to initially administer ceftazidime, meropenem or imipenem for 10-14 days followed by prolonged oral eradication therapy with trimethoprim-sulfamethoxazole for 3-6 months is recommended. Doxycycline or amoxicillin-clavulanate are alternatives for allergic individuals.

D. Post-exposure prophylaxis for laboratory workers is recommended following exposure to both organisms. Based on animal data, the Center of Diseases and Control recommend oral agents over a three week period. Trimethoprim/sulfamethoxazole is the drug of choice (or amoxicillin-clavulanate or doxycycline, if allergic)

VIII. BT Agent Clinical Summary

A.

Clinical Information	*B. mallei*	*B. pseudomallei*
Virulence Factor	Little studied, possible antiphagocytic capsule	Possibly LPS, exotoxin intracellular survival, antiphagocytic capsule
Infective Dose	Low	Low
Incubation Period	10-14 days via aerosol	2 days to 26 yr
Duration of Illness	Death 7-10 days in septicemic form	Days to months
Person to Person Transmission	YES (low)	YES (rare)

B. Cases of laboratory-associated melioidiosis have been reported sporadically.
1. The risk of infection is presumably greater for workers in areas where the disease is not endemic, because lack of experience working with *B. pseudomallei.*
2. The primary hazards for lab workers arise from direct contact with cultures and infectious samples and from exposure to infectious aerosols.

IX. Quality Control

Refer to individual culture procedures in the Bacteriology Manual.

X. References

A. **CDC** Website information. Disease Information: Glanders (*Burkholderia mallei*).
B. **CDC** Website information. Disease Information: Melioidosis (*Burkholderia pseudomallei*).
C. **Murray, P.,** et al. 2003. Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.
D. **Gilligan, P.H. and York, M.K**. 2013. Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism. *Burkholderia mallei* and *B. pseudomallei*. ASM.
E. **Sharp, S.E.** 2010. Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases. Clinical Laboratory Bioterrorism Readiness Plan. ASM.

XI. Attachments

A. Guidelines for GPR and GNR BT flowcharts
B. Gram Negative Rod Select Agent Identification Chart

XII. Revision Record

A. 5/2014
1. Streamlined testing information; included new tests (Amoxicillin-Clavulanic acid (20/10µg) and penicillin (10U)); removed TSI test
2. Added section BT Agent Clinical Summary
3. Revised Section Interpretation and Reporting

B. 3/2015
1. Section V. D. Added Bruker library does not contain select agents
2. Section V. D. Added list of organisms that can be misidentifications
3. Section XI. Discontinued *B. mallei/B.pseudomallei* flowcharts. Added new consolidated chart.

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Clinical Microbiology Laboratory Document # 605.U.106.06

Bioterrorism Manual **Procedure for Rule Out of *Clostridium botulinum***		Effective:
Written by: Karen LaFé	Reviewed by: Sarah Jensen	Approved by: Brad Cookson
Revises or supersedes: Same of 3/31/14		Revised by: Lynda Bui

ANNUAL REVIEW			
Reviewed by:	Date	Reviewed by:	Date

I. Principle

Clostridium botulinum is a group of anaerobic organisms, commonly found in soils and aquatic habitats throughout the world, that are alike only in that they are clostridia and produce antigenically distinct neurotoxins with similar pharmacologic actions. The seven types of *C. botulinum* (A to G) are distinguished by the antigenic characteristics of the neurotoxins they produce. Human botulism is primarily caused by the strains of *C. botulinum* that produce toxin types A, B, and E, but rare cases of type F have been reported. Four distinct forms of botulism have occurred in humans: foodborne, wound, infant and child or adult non-foodborne.

The suspicion of botulism is a public health emergency. *C. botulinum* toxin has been classified as a possible agent of bioterrorism because it is extremely potent and lethal. The UWMC Clinical Microbiology Laboratory WILL NOT ACCEPT specimens for "R/O Botulism" or "R/O" or "Culture for *Clostridium botulinum*" nor will it attempt to culture or identify the organism. Notify both local public health officials and the Washington State Public Health Laboratory for approval to submit samples for testing. Specimens for these requests should be forwarded without delay to the state public health laboratory when approval is granted.

Unsuspected *Clostridium botulinum* isolates may be recovered during routine culture workup. See the following procedure for description of workup.

II. Set-up Procedure

No specimen will be processed for requests of **"R/O Botulism" or "R/O" or "Culture for *Clostridium botulinum*"**. Consult with BT tech/manager for further action. The state lab will need to be notified. Transfer of any specimens will need to be approved.

III. Gram Stain, Colony and Biochemical Characteristics

Unsuspected isolation of a potential *C. botulinum* should be handled as outlined below.

Note: Handle all isolates in the biosafety cabinet. Use resealable plastic bags to enclose plates and use disposable sterile sticks and loops as needed.

A. Gram stain
   1. Large, anaerobic, gram-positive rods.
   2. Oval, subterminal spores which swell the cell.

B. Colony
   1. Anaerobic growth at 35°C in 24-30 hrs.
   2. Beta hemolytic with rhizoid colonies on moisture-free media, swarming colonies on damp media.

C. Biochemicals
   1. Catalase negative

2. Indole negative
3. Lipase positive

IV. Interpretation and Reporting

A. ***Clostridium sporogenes* is phenotypically the same as *C. botulinum* and will identify on the mass spec. *C. sporogenes* is a *C. botulinum* without the toxin genes.**

B. **Consult with the BT tech/lead. Notify the clinical microbiology director/fellow to review patient history.**
   1. If the patient has **NO** clinical presentations, then report as "Presumptive *Clostridium sporogenes*".
   2. If the patient has clinical presentation, treatment and testing coordination must be made with the state lab and epidemiologist by the clinican.

C. Refer to the procedure "Select Agent Handling and Bioterrorism Preparedness" for instructions on notifications and federal requirements. The isolate will need to be sent to the WSPHL.

D. If *C. botulinum* is ruled out, proceed with efforts to identify using established procedures.

V. Antimicrobial Susceptibility Testing

The administration of antitoxin is the only specific therapy available for botulism and evidence suggests that it is effective only if given very early in the course of neurologic dysfunction.

VI. BT Agent Clinical Summary

Source: "Sentinel Level Clinical Laboratory Guidelines: Clinical Laboratory Bioterrorism Readiness Plan, Appendix E"

A. Virulence: Neurotoxin

B. Infective dose: 50% lethal dose 0.001 g/kg of body weight

C. Incubation period: 6 hrs to 10days (usually 1-5 days)

D. Duration of illness: Death in 24-72hrs; lasts months if not lethal

E. Person to Person transmission: No

F. Botulism toxins are extremely poisonous. Exposure to the toxin by ingestion, inhalation, or absorption can cause intoxication and death.

VII. Quality Control

Refer to individual culture procedures in the Bacteriology Manual.

VIII. References

A. **Murray, P.R**., et al. 2007. Manual of Clinical Microbiology, ASM Press, Washington, D.C.

B. **Shiflett, S.L. and Robinson-Dunn, B.** et al. 2013. Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism. Botulinum Toxin. ASM, Washington, D.C.

C. **Garcia, L.** 2010. Clinical Microbiology Procedures Handbook. Bioterrorism: Botulinum Toxin. ASM Press, Washington. D.C.

D. **Washington State Public Health Laboratory.** 2015. *Clostridium* issue. (How to handle *C. sporogenes* isolates.) Email communication with Dr. Jacky Chow, Clinical Microbiology Fellow and Dr. William Glover, WSPHL Microbiology Supervisor. Shoreline, WA.

IX. Revision Record

A.	3/2012	Section I, amended instructions for accepting samples for botulinum testing. Section IV, added instructions for how to handle an isolate for toxin testing. Section IX, added reference communication.
B.	3/2014	Section I, no samples will be tested and must be sent to WSPHL. Section IV, added statement *C. sporogenes* is phenotypically the same as *C. botulinum*
C.	3/2015	Section IV, added involvement of laboratory director or fellow to determine whether patient's clinical presentation requires additional testing. State lab will perform testing only if symptomatic patient.

University of Washington Medical Center
Clinical Microbiology Laboratory Document # 605.U.107.05

Bioterrorism Manual **Procedure for Rule Out of *Francisella tularensis***		Effective:
Written by: Karen LaFé	Reviewed by: Sarah Jensen	Approved by: Brad Cookson
Revises or supersedes: Procedure for Culture of Bioterrorism Agents: *Francisella tularensis* of 5/5/14		Revised by: Lynda Bui

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

I. Principle

Francisella tularensis is an extremely infectious organism which is widespread in nature. It infects primarily wild animals. Ticks, mosquitoes and biting flies have been implicated as vectors of tularemia bacteria that infect animals and humans. Contaminated hay, water, infected carcasses, chronically infected animals, and aerosolized particles have been documented as sources of infection. *F. tularensis* is one of the most infectious bacteria known and can cause severe illness and death in humans.

All specimens and cultures should be processed and examined with care in a biological safety cabinet. Every precaution should be taken to avoid the production of aerosols of the infected material. Once definitive identification of *F. tularensis* is known, the isolate is to be handled only by designated personnel.

II. Specimens

Acceptable specimens include blood culture, biopsied tissue or scraping of an ulcer, aspirate of involved tissue and pleural fluid.

III. Specimen Transport

Transport the specimen to the laboratory without delay.

IV. Set-up Procedure

A. Refer to the "Procedure for Culture Processing of Bioterrorism Agents and Campus Health Requests for RCE Workgroup Exposure Specimens" in the Setup and Bioterrorism Manuals.

B. Use established inoculation and plating procedures. Tape plates shut or enclose in plastic baggies to prevent inadvertent opening.

C. Incubation
   1. Temperature: 35-37°C.
   2. Atmosphere: ambient, use of 5% CO_2 is acceptable.
   3. Length of incubation:
      a. Blood cultures and fluid cultures in blood culture bottles: incubate for 10 days. Perform terminal Acridine orange stain and subculture to CHOC and BRU at day 10. Hold subcultures 4 days.
      b. Tissues: Incubate plates for 3 days, broths for 7 days.

V. Identification

A. Gram stain
   1. Characteristics: staining of *F. tularensis* often reveals the presence of tiny, 0.2-2.0.5 µm X 0.7-1.0 µm, pleomorphic, faintly staining, gram-negative coccobacilli seen mostly as single cells. The gram stain interpretation may be difficult because the cells are minute and poorly staining.
   2. *F. tularensis* cells are smaller than *Haemophilus influenzae*. Bipolar staining is not a distinctive feature of *F. tularensis* cells.

B. Colony characteristics
   1. *F. tularensis* grows in commercial blood culture media.
   2. These organisms require cysteine supplementation; therefore, *F. tularensis* may at first grow on BA, but upon subsequent passage will fail to grow on standard BA.
   3. On cysteine supplemented agar plates (CHOC), it is a gray-white, opague colony, usually too small to be seen at 24 hrs on most general media such as CHOC, TMA and BCYE. After incubation for 48 hrs or more, colonies are about 1-2 mm in diameter, white to gray to bluish-gray, opaque, flat with an entire edge, smooth and have a shiny surface. *F. tularensis* will not grow on MAC.
   4. **As soon as F. tularensis is suspected, perform all further work in a biosafety cabinet.**

C. **WARNING**
   1. Automated identification systems may key out as non-*F. tularensis* (e.g. *Haemophilus influenzae* and *Aggregatibacter (includes previous Actinobacillus)).*
   2. The Bruker mass spectrometry library DOES NOT contain bioterrorism agents. Results may be reported as "no reliable information".
   3. **Organisms to consider as potential look-a-likes:**
      a. ***Haemophilus influenzae***
      b. ***Brucella* sp.**
      c. ***Pasteurella multocida***

D. Testing and Observation in the BSC
   1. Gram stain: faint staining tiny gram negative coccobacillus
   2. MAC: No growth
   3. BAP: Scant growth
   4. CHOC: Better growth than BAP, grayish white colonies
   5. Oxidase: negative
   6. Catalase: negative (or weakly positive)
   7. Satellite growth: negative
   8. Beta lactamase: positive
   9. Urea: Negative (Not part of current ASM guidelines; compare to *Brucella* which is positive with some strains delayed 72hrs)

VIII. Interpretation and Reporting

A. Any isolate where *F. tularensis* cannot be ruled out should be brought immediately to the attention of the director, lab manager, and BT Techs/lead.

B. If it has been determined that the isolate must be sent for identification, report "X+ Gram negative rods: Identification to Follow. Sent to State Lab for testing (STSL)"

C. **Follow the notification instructions and regulatory guidelines outlined in the procedure "Select Agent Reporting and Bioterrorism Preparedness".**

D. If the organism is determined to be a select agent (either by the State Lab or the molecular section), refer to the above procedure for reporting.

E. **Secure all culture media and specimen(s). Hand over to the BT tech.**

IX. Antimicrobial Susceptibility Testing

A. Susceptibility testing is not performed by this laboratory.

B. *F. tularensis* infections are treatable with narrow-spectrum antibiotics. All *Francisella* isolates examined to date are β-lactamase positive, so penicillins and cephalosporins are not effective and should not be used to treat tularemia.

C. Post-exposure prophylaxis recommendations for laboratory workers should be referred to the infectious disease service and exposure reported to occupational health.

X. BT Agent Clinical Summary

Source: "Sentinel Level Clinical Laboratory Guidelines: Clinical Laboratory Bioterrorism Readiness Plan, Appendix E"

A. Virulence factor: Intracellular survival

B. Infective dose: 10-50 organisms

C. Incubation period: 2-10 days

D. Duration of illness: ≥2 weeks

E. Person to person transmission: Single case report during autopsy

F. Isolation precautions for hospitalized: Standard

G. Laboratory hazards include direct contact of skin or mucous membranes with infectious material, accidental parenteral inoculation or ingestion, and exposure to infectious aerosols or droplets through manipulation of cultures.

XI. Quality Control

Refer to individual culture procedures in the Bacteriology Manual.

XII. References

A. **Craft, D. ,** et al. 2014. Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Francisella tularensis*. ASM Press, Washington. D.C.

B. **Murray, P.,** et al. 2003 Manual of Clinical Microbiology, 8th ed. ASM Press, Washington, D.C.

C. **Weyant, R.,** et al. 1996. Identification of Unusual Pathogenic Gram-Negative Aerobic and Facultatively Anaerobic Bacteria, 2nd ed. Williams and Wilkins, Baltimore, Maryland.

D. **Patel, R. et al** 2013 Importance of Using Bruker's Security-Relevant Library for Biotyper Identification of *Burkholderia pseudomallei*, *Brucella* species and *Francisella tularensis*. Journal of Clinical Microbiology, Volume 51, Number 5, p. 1639-1640.

E. **State of Washington Department of Health, 2014**. Public Health Laboratory Sentinel Training Program

XIII. Attachments

A. Guidelines for GPR and GNR BT flowcharts
B. Gram Negative Rod Select Agent Identification Chart

XIV. Revision Record

A. 5/2014: Removed urea testing and added section "BT Clinical Summary"

B. 3/2015:
Section V. C. Added warning regarding mass spectrometry use
Section V. D. Added comparison of BA and CHOC per latest ASM guidelines, replacing 2013 version
Section V. D. Added urea testing for rule out of *Brucella*; also recommended by state LRN sentinel training program
Section XIII. Discontinued "Rule Out *Francisella tularensis* BT Algorithm #5: Gram Negative Bacilli or Coccobacilli". Added new consolidated chart.

University of Washington Medical Center
Clinical Microbiology Laboratory Document # 605.U.108.05

Bioterrorism Manual **Procedure for Rule Out of *Yersinia pestis***		Effective:
Written by: Karen LaFé	Reviewed by: Sarah Jensen	Approved by: Brad Cookson
Revises or supersedes: Procedure for Culture of Bioterrorism Agents: *Yersinia pestis* of 10/20/2009, 5/5/2014		Revised by: Lynda Bui

ANNUAL REVIEW			
Reviewed by:	Date	Reviewed by:	Date

I. Principle

Yersinia pestis is a virulent organism which causes infections in animals and humans. The causative agent of plague, it has a protracted history, being described in epidemics and pandemics since biblical times. Sylvatic plague is endemic in 11 western U.S. states. It is carried by squirrels, chipmunks, wood rats, marmots, wild pigs, bears, and occasionally by hares and rabbits. Humans can acquire plague through the bite of infected fleas, direct contact with contaminated tissue, or inhalation. Clinically, plague may present in bubonic, septicemic and pneumonic forms. The organism can occasionally be passed from human to human by close contact as in primary pneumonic plague. Primary pneumonic plague is most likely the form that would be seen if *Y. pestis* were used in a bioterrorism event. This is due to the high likelihood of aerosol delivery; however the communicability of this form of the disease would make control of this particular agent even more problematic.

All specimens and cultures should be processed and examined with care in a biological safety cabinet. Every precaution should be taken to avoid the production of aerosols of the infected material. Once definitive identification of *Y. pestis* is known, the isolate is to be handled only by designated personnel.

II. Specimens Collection

Clinical specimens acceptable for culture are:

A. Lower respiratory tract (pneumonic)
B. Blood (septicemic)
C. Aspirate of involved tissue (bubonic) or biopsied specimen

III. Specimen Transport

Transport specimens in a trans-envelope with requisition. Specimens should be received as soon as possible after collection for optimal recovery.

IV. Set-up Procedure

A. Refer to the "Procedure for Culture Processing of Bioterrorism Agents and Campus Health Requests for RCE Workgroup Exposure Specimens" in the Setup and Bioterrorism Manuals.

B. All patient specimens should be handled while wearing gloves and gowns and working in a biosafety cabinet. Subcultures should be performed in a BSC and incubated in 5-10% CO_2. Plates should be taped shut or enclosed in plastic baggies and all further testing should be performed only in the BSC.

C. Incubation

1. Temperature: 28-30°C (optimal); 35-37°C (grow more slowly).
2. Atmosphere: Ambient, use of 5% CO_2 is acceptable.
3. Length of incubation: Hold primary plates for 5 days. Plates should be held for up to 7 days if the patient has been treated with bacteriostatic antibiotics.

V. Identification

A. Gram stain

Characteristics: Direct microscopic examination of specimens and cultures by Gram stain can provide a rapid presumptive identification. Stained specimens containing *Y. pestis* often reveal plump, gram-negative rods, 1-2 µm X 0.5 µm, that are seen mostly as single cells or pairs and short chains in liquid media.

B. Wright or Giemsa Stains

Although not normally performed in the microbiology laboratory, Wright or Giemsa stains performed on peripheral blood or tissue by hematology or histopathology may reveal the bipolar staining characteristics of *Y. pestis*, whereas the Gram stain may not. Patients with overwhelming sepsis, bipolar staining rods may be detected in peripheral blood smears. It is useful to note these characteristics in the event that hematology or histopathology asks for a microbiology consult.
(Note: This feature can also be seen in other *Yersinia* sp, enteric bacteria, and other gram negative organisms particularly *Pasteurella*.)

C. Colony characteristics

Y. pestis grows as grey-white, translucent colonies, usually too small to be seen as individual colonies at 24hrs. After incubation for 48hr, colonies are approximately 1-2mm in diameter, gray-white to slightly yellow, and opaque. There is little or no hemolysis of the sheep red blood cells. *Y. pestis* will grow as small, non-lactose fermenting colonies on MAC.

D. **WARNING**

1. Automated identification systems often key out *Y. pestis* as non-*Y. pestis* (e.g. *Shigella*, H_2S-negative *Salmonella*, *Acinetobacter*, *Pseudomonas* and *Yersinia pseudotuberculosis*).
2. The Bruker mass spectrometry library DOES NOT contain bioterrorism agents. Results may report out as "***Yersinia pseudotuberculosis***".

E. Testing and Observation in the BSC

1. Pinpoint colony at 24 hrs on BA.
3. Non-lactose fermenter, may not be visible on MAC at 24 hrs.
4. Oxidase negative
5. Catalase positive
5. Urease negative
6. Indole negative
7. Growth at 25°C and 35°C

F. Differentiation of Other Important *Yersinia* species

***Yersinia* species**	**Oxidase**	**Catalase**	**Urea**	**Indole**
Y. pseudotuberculosis	Negative	Positive	Positive	Negative
Y. enterocolitica	Negative	Positive	Positive	Variable
Y. frederiksenii	Negative	Positive	Positive	Positive
Y. kristensenii	Negative	Positive	Positive	Variable
Y. ruckeri	Negative	Positive	Negative	Negative
Y. pestis	**Negative**	**Positive**	**Negative**	**Negative**

VI. Interpretation and Reporting

A. Any isolate where *Y.* pestis cannot be ruled out should be brought immediately to the attention of the director, lab manager, and BT Techs/lead.

B. If it has been determined that the isolate must be sent for identification, report "X+ Gram negative rods: Identification to Follow. Sent to State Lab for testing (STSL)"

C. **Follow the notification instructions and regulatory guidelines outlined in the procedure "Select Agent Reporting and Bioterrorism Preparedness".**

D. If the organism is determined to be a select agent (either by the State Lab or the molecular section), refer to the above procedure for reporting.

E. **Secure all culture media and specimen(s). Hand over to the BT tech.**

VII. Antimicrobial Susceptibility Testing

A. Antimicrobial susceptibility testing of *Y. pestis* is not performed by this laboratory.

B. Refer to the WSPHL for testing is a physician requests it.

VIII. Quality Control

Refer to individual culture procedures.

IX. BT Agent Clinical Summary

Source: "Sentinel Level Clinical Laboratory Guidelines: Clinical Laboratory Bioterrorism Readiness Plan, Appendix E"

Pneumonic Plague

A. Virulence factor: V and W antigens, Lipopolysaccharide (endotoxin), F1 antigen
B. Infective dose: <100 organisms
C. Incubation period: 2-3days
D. Duration of illness: 1-6 days
E. Person to person transmission: YES (high)
F. Isolation precautions for hospitalized: Droplet
G. Primary hazards to laboratory personnel are direct contact with cultures and infectious materials, autoinoculation or ingestion, and exposure to aerosols or droplets produced by manipulation of cultures.

X. References

A. **Isenberg, H**. 2004. Clinical Microbiology Procedures Handbook. ASM Press, Washington, D.C.

B. **Murray, P**. et al., 2003. Manual of Clinical Microbiology 8th ed., ASM Press, Washington, D.C.

C. **Sharp, S.E**., et al., 2014. Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism, *Yersinia pestis.* ASM Press, Washington. D.C.

XI. Attachments

A. Guidelines for GPR and GNR BT flowcharts
B. Gram Negative Rod Select Agent Identification Chart

XII. Revision Record

A. 5/2014: Added section BT Agent Clinical Summary

B. 5/2014: Added *Yersinia* table of other important species

C. 3/2015: Reorganized flow; Section V. A. removed “B-Safe BT Bench Aid”; Section V. D. added warning regarding mass spec library; Section XI. discontinued flowchart for combined BT GNR chart

University of Washington Medical Center
Clinical Microbiology Laboratory Document # 605.U.314.03

Bioterrorism Manual **Guidelines for GNR and GPR BT Flowcharts**		Effective: 3/2015
Process Document	Written by: Susan Turner	Reviewed by: Susan Turner
Revises or supersedes: Same of 1/3/11, 3/2014		Revised by: Lynda Bui

ANNUAL REVIEW			
Reviewed by:	Date	Reviewed by:	Date

I. Purpose

This document briefly summarizes preliminary work to rule out possible select agents. Refer to the Bioterrorism manual for complete procedures.

II. Gram Negative Rod Workup

This document is to be used in conjunction with "Gram Negative Rod Select Agent Identification Characteristics" chart.

A. From growth on original media: Sub to BA, MAC, CHOC. Wait 48 hours before determining which media the organism does/does not grow on.

B. Perform all testing in BSC. Place all bags all plates.

C. Start with oxidase and catalase. (**Wait for 48hrs of growth prior to performing the oxidase test.)**

1. Oxidase pos/Catalase pos: Think *Brucella, B. mallei,* or *B. pseudomallei*

2. Oxidase neg/Catalase pos: Think *Francisella, B. mallei,* or *Yersinia pestis*
   Is catalase weak? If yes, think *Francisella.*

3. Set up each of the following tests.

TESTS TO SETUP
Spot indole
BAP (RT/35°/42°C)
Satellite Test with *S. aureus*
Beta Lactamase (Setup if thinking *Francisella)*
Urea
Motility (35°C)
BT disks on MH, 0.5 McF: **colistin, amoxicillin-clavulanate, penicillin**
Arginine

4. In the micro workup, use workup group code "WBTGNR" to bring up all of the tests setup above.

D. During the course of identification, organisms may look like others biochemically or morphologically. Be aware of these misidentifications as highlighted below. These are more reflected with commercial systems (e.g. Vitek). Mass spectrometry results are mentioned where applicable.

Organism	Misidentified as
Brucella	*Haemophilus influenzae, Francisella tularensis, Oligella ureolytica, Ochrobactrum anthropi*
Francisella	*Acinetobacter, Brucella, H. influenzae, Pasteurella multocida*
Burkholderia mallei	*??*
Burkholderia pseudomallei	*B. cepacia, B. oklahomensis (MS), B. thailandensis (MS), Chromobacterium violaceum*
Yersinia pestis	*Y. pseudotuberculosis (MS)*

MS = mass spectrometry identification

E. Clinical data, if available, may help identification.

1. *Brucella*: Primarily found in cattle, swine, sheep and goats. Usually transmitted to humans through skin abrasions, consumption of contaminated food products (for example unpasteurized goat milk), or inhalation. Symptoms include fever (often undulating), headache, malaise, muscle aches and anorexia.

2. *Francisella*: Associated with a variety of animals, primarily hares, rabbits and rodents. Can be transmitted to humans from tick or deer fly bites, from contact with infected animals (for example skinning rabbits) or from contact with pets (dogs, cats, hamsters, prairie dogs) and zoo animals. Primarily a rural disease. Symptoms include fever, chills, headache, and malaise.

3. *Burkholderia mallei*: Is the cause of glanders in livestock, particularly horses, mules and donkeys. Primarily found in the Middle East, Asia, Africa and South America. Has been eradicated from most countries.

4. *Burkholderia pseudomallei*: Primarily found in Southeast Asia, tropical northern Australia; also can be found in the Indian subcontinent, and in Central and South America. These organisms should be considered with fever of unknown origin or a tuberculosis-like illness with history of travel to a region where these organisms are endemic.

5. *Yersinia pestis*: Found in rodents. Causes plague (pneumonic, septicemic and bubonic plague). The most common clinical presentation is bubonic plague. Symptoms include fever and painful swellings (bubos) in the area of the infected lymph node. Transmission to humans is associated with the rat flea. The most common reservoir in the U.S. for transmission is the squirrel. A secondary mode of transmission is contact with infected cats (who eat contaminated rodents) by bites or scratches. Most cases of plague occur in New Mexico, Arizona, Colorado, and California.

III. Gram Positive Rod Workup

This document is also to be used in conjunction with GPR BT flowchart 605.U.307. Additionally, there is no flowchart for GPR *Clostridium botulinum* since it is the toxin producing organism that is the select agent. This document will summarize some details to utilize for rule out identification.

A. If it is an **aerobic** gram positive rod, use the rule out *B. anthracis* BT flowchart.

B. If it is an **anaerobic** boxy gram positive rod, consider *Clostridium* species.

1. **Note: When there is a clinical suspicion of botulism, testing will be arranged with the state lab for toxin testing. Therefore, no culture testing is performed here in the laboratory.**

The laboratory may encounter *Clostridium sporogenes*, which looks like *C. botulinum* and will identify on the mass spec since they are closely related. *C. sporogenes* is a *C. botulinum* without the toxin genes. Consult with the BT tech/lead. The micro fellow/director will review patient history to determine additional testing.

2. Evaluate for hemolysis, catalase, indole, and lipase activity. Refer to the procedure for *C. botulinum.*

Gram Negative Rod Select Agent Identification Chart

Use this chart with "Guidelines for GNR and GPR BT Identification" and the procedures for each organism.
Perform all tests and subcultures in the BSC.

Tests	*Brucella*	*Francisella*	*B. mallei*	*B. pseudomallei*	*Y. pestis*
GRAM	GNCB, stains poorly; does not cluster	GNCB, pleomorphic, faintly staining	GNCB, small, straight/slightly curved	GNR, small, straight/slightly curved	GNR, plump
BAP35	Poor growth 24hrs, small white at 48hrs (CAUTION: can be mistaken for Staph)	Scant/NG >48hrs	Poor growth 24hrs; small gray at 48hrs; no pigmt on Mueller Hinton	Poor grwth at 24hrs, good grwth white at 48hrs; can be dry wrinkly with age; no pigmt on Mueller Hinton	Pinpoint gray white/opaque after 24hrs; **growth on BA room temp**
BAP42			NG	Growth	
MAC	NG	NG 48hrs	Poor/NG 48hrs	NLF 48hrs; can look pink but not fermenter	NLF; scant at 24hrs
CHOC	Poor growth 24hrs, small white at 48hrs (CAUTION: can be mistaken for Staph)	Small gray white on CHOC >48hrs	Growth	Growth	Growth
OX	+	-	VARIABLE (MOSTLY NEGATIVE)	+	-
CAT	+	- or weak +	+	+	+
BETA LACTAMASE		+			
SATELLITE	-	-			
SPOT INDOLE			-	-	-
UREA	+; some strains delayed 72hrs	-			-
ARG			+	+	
MOTILITY	-	-	-	+	-
COLISTIN* Interpretation: Any zone is susceptible			No zone	No zone	
AMOX-CLAV* Interpretation: ≥18mm=susceptible			Susceptible	Susceptible (Rare resistance)	
PEN* Interpretation: ≤28mm = resistant ≥29mm=susceptible			Resistant	Resistant	

*Perform using the Kirby-Bauer disk diffusion method with 0.5 McFarland standard. If it does not grow on MH, BAP or CHOC can be used solely for identification.