University of Washington Medical Center
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Bioterrorism Manual **Procedure for Rule Out of *Bacillus anthracis* and *Bacillus cereus* biovar anthracis**		Effective: 2/26/18
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Supersedes: Procedure for Culture of Bioterrorism Agents: *Bacillus anthracis* of 11/03/2010, 3/31/14, 4/2015, 7/17/17		Revised by: Lynda Bui

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.

Bacillus cereus biovar *anthracis* has only been found in certain African countries, including Cameroon and Cote d'Ivoire. These strains are known to cause an anthrax-like disease in gorillas and chimpanzees, and have been isolated from other animals, including elephants and goats. *B. cereus* biovar *anthracis* strains are genetically similar to *B. anthracis* and produce all of the primary *B. anthracis* virulence factors, thus they are now considered to be select agents in the United States.

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* or *Bacillus cereus* biovar *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 document "Culture Processing of Bioterrorism Agents" 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* or *Bacillus cereus* biovar *anthracis* may be observed as early as 8 hrs after incubation.

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V. Identification

A. Stains and Smears: Gram Stain

1. *B. anthracis* and *Bacillus cereus* biovar *anthracis* are large gram-positive rods (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* and *Bacillus cereus* biovar *anthracis*

B. anthracis and *Bacillus cereus* biovar *anthracis* grow 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. **See the "Select Agent Images" document in the Bioterrorism Manual for pictures of the colonies.**

C. Testing and Observation in the BSC

1. Growth on BA: White colonies with Ground glass appearance and may have "Medusa head" characteristics (comma-shaped, edges that are slightly undulate). Colonies are tenacious and when teased with a loop the growth will stand up like beaten egg whites.
2. Hemolysis: Nonhemolytic on BA (**BEWARE: *B. cereus* biovar *anthracis* can exhibit weak hemolysis upon extended incubation (48hrs), particularly in CO_2.)**
3. Growth on MAC: no growth
4. Catalase: positive
5. Motility in semi-solid medium: *Bacillus anthracis* usually negative; *B. cereusi* biovar *anthracis* are usually motile
   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.
6. ***Bacillus anthracis* and *Bacillus cereus* biovar *anthracis* is ruled out if the catalase test is negative or the isolate is strongly beta hemolytic at 24hrs or the colony is mucoid.**

VI. Interpretation and Reporting

A. **The Bruker MALDI-TOF MS can misidentify anthrax as any of the species in the *Bacillus cereus* complex (***B. cereus, B. mycoides, B. pseudomycoides, B. thuringiensis, B. weihenstephanensis.* **Therefore, any non-hemolytic gram positive rod identified as any of these species should be ruled out as *B. anthracis* or *B. cereus* biovar *anthracis* by colony morphology.**

B. Any isolate that cannot be ruled out as *B. anthracis* or *B. cereus* biovar *anthracis* should be brought immediately to the attention of the lab manager and BT tech/lead. The micro fellow or director will be consulted to look up the patient's history and/or contact the clinical team.

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

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

E. **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, the request will be referred to WSPHL.

C. Quinolones (e.g. ciprofloxacin, levofloxacin), doxycycline, and penicillin are currently the only FDA-approved antibiotics for the treatment of anthrax (Center of Biosecurity of UPMC).

D. Post-exposure prophylaxis for personnel (adults and children), including laboratory personnel suspected of exposure to *B. anthracis* or *B. cereus* biovar *anthracis* spores include ciprofloxacin, levofloxacin (adults) or doxycycline (Center of Biosecurity of UPMC).

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* and *B. cereus* biovar *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. **Mahlen, S.D. and Snyder J.W.** 2017 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. 4/2015: Reorganized for flow; Section V. C. removed 25°C motility; replaced *Bacillus* flowchart
E. 7/2017: Section V. B. Added statement to refer to "Select Agent Images" document for pictures of anthrax colonies
Section IV. Added statement that anthrax can be misidentified as species of the *Bacillus cereus* complex by mass spec.
F. 2/2018: Document update to include *Bacillus cereus* biovar *anthracis* in testing, interpretation, susceptibility information, new reference