

Addition of *Bacillus cereus* biovar *anthracis* as a Tier 1 Select Agent

Background: Effective October 14, 2016, the Centers for Disease Control and Prevention (CDC) in the Department of Health and Human Services (HSS) added *Bacillus cereus* biovar *anthracis* as a Tier 1 select agent to the HSS list of select agents and toxins (1).

Organism information: *B. cereus* biovar *anthracis* was first described as an agent of anthrax-like disease in gorillas and chimpanzees in Cameroon and Côte d’Ivoire (2). The organism has since been recovered from an elephant and goats in other countries of Africa (3). The CDC has determined that *B. cereus* biovar *anthracis* has all of the virulence determinants and the biothreat potential of *B. anthracis* (1). *B. cereus* biovar *anthracis* isolates are non-hemolytic, like *B. anthracis*, and motile, like *B. cereus*. The characteristics of *B. cereus* biovar *anthracis* compared to *B. anthracis* and *B. cereus* are summarized in the following table (adapted from references 1 and 2):

Characteristic	<i>B. anthracis</i>	<i>B. cereus</i>	<i>B. cereus</i> biovar <i>anthracis</i> CI Primary ¹	<i>B. cereus</i> biovar <i>anthracis</i> CI Sub ²	<i>B. cereus</i> biovar <i>anthracis</i> CA Primary ³	<i>B. cereus</i> biovar <i>anthracis</i> CA Sub ⁴
Hemolysis	-	+	-	+/- ⁵	-	+/-
Motility	-	+	+	+	+	+
Gamma phage susceptibility	+	-	-	+/-	-	+/-
Penicillin G ⁶	S	R	S	S/R	R	R
Capsule	+	Absent in vitro	+	+/-	+	+/-

- 1: CI = Côte d’Ivoire strains, primary culture
- 2: Côte d’Ivoire strains, subculture
- 3: CA = Cameroon strains, primary culture
- 4: Cameroon strains, subculture
- 5: +/- = some subclones positive, others negative
- 6: S= susceptible; R = resistant

Challenges: Based on the organism characteristics described above and the limited number of strains available for study, a sentinel laboratory protocol using rapid rule-out or refer tests to differentiate *B. cereus* biovar *anthracis* from other *Bacillus* spp. is currently not available. Subject matter experts at the Centers for Disease Control and Prevention (CDC), the American Society for Microbiology (ASM), and the Association of Public Health Laboratories (APHL) are working to develop testing algorithms for *B. cereus* biovar *anthracis*.



Recommendations: Sentinel laboratories should continue using the existing *ASM Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases, Bacillus anthracis* (<http://www.asm.org/images/PSAB/LRN/Anthrax316.pdf>) to rule-out or refer isolates of *Bacillus* spp. that produce non-hemolytic colonies with a ground glass appearance and are non-motile. Until new guidelines are available, the following recommendations should be considered:

- 1) Suspect *Bacillus* spp. isolates that are large Gram-positive rods and weakly or non-hemolytic (at 24 hours or less) should be tested for motility and catalase production. Semi-solid medium is recommended for motility for consistent results.
- 2) Isolates that are positive for catalase and motility should be investigated further by contacting the patient's attending physician to determine if the patient has an anthrax-like illness or if the patient has an infection caused by this organism. If the isolate is deemed significant, the local LRN reference laboratory (e.g. state or local public health laboratory) should be contacted and the isolate forwarded for further testing.
- 3) If the sentinel laboratory is unable or unwilling to contact the patient's physician, notify the local LRN reference laboratory and provide the physician's contact information and laboratory testing results.

References:

- 1) Federal Register, Wednesday, September 14, 2016, **81**:63138-63143. Also available at: <https://www.federalregister.gov/documents/2016/09/14/2016-22049/possession-use-and-transfer-of-select-agents-and-toxins-addition-of-bacillus-cereus-biovar-anthraxis>.
- 2) Klee, S. R., M. Özel, B. Appel, C. Boesch, H. Ellerbrok, D. Jacob, G. Holland, F. H. Leendertz, G. Pauli, R. Grunow, and H. Nattermann. 2006. Characterization of *Bacillus anthracis*-like bacteria isolated from wild great apes from Côte d'Ivoire and Cameroon. *J. Bacteriol.* **188**:5333-5344.
- 3) Antonation, K. S., K. Grützmacher, S. Dupke, P. Mabon, F. Zimmermann, F. Lankester, T. Peller, A. Feistner, A. Todd, I. Herbinger, H. M. de Nys, J.-J. Muyembe-Tamfun, S. Karhemere, R. M. Wittig, E. Couacy-Hymann, R. Grunow, S. Calvignac-Spencer, C. R. Corbett, S. R. Klee, and F. H. Leendertz. 2016. *Bacillus cereus* biovar *anthracis* causing anthrax in Sub-Saharan Africa—chromosomal monophyly and broad geographic distribution. *PLoS Negl. Trop. Dis.* **10**:1-14.