



A NEW TIER 1 SELECT AGENT—*CEREUS-LY!*

Leah Kloss*

Tier 1 select agents are believed to pose the greatest threat for deliberate misuse and potential harm to public health; therefore, they are subject to the highest level of biosafety and security regulations, and have the most limitations on possession and transfer. Effective October 14, 2016, the Centers for Disease Control and Prevention (CDC) in the Department of Health and Human Services (HHS) added *Bacillus cereus* biovar *anthracis* as a Tier 1 select agent to the HHS list of select agents and toxins.¹

As you might guess from its name, it is a *Bacillus cereus* that can produce an anthrax-like disease. Animal models suggest that it is transmissible to humans by the same routes as *B. anthracis*.² Because of its similarity to *B. anthracis*, CDC and HHS have decided that it poses the same category of risk to the public's health and national security, and added it to the Tier 1 subcategory of the select agent list.³

B. cereus is a gram-positive, aerobic, spore-forming rod, commonly found in the environment. It is an opportunistic pathogen known to cause foodborne illnesses in humans. The *B. cereus* biovar has plasmids that are genetically like those found in *Bacillus anthracis* and produce the same virulence determinants – edema, lethal toxins, and a capsule. *B. cereus* biovar *anthracis* isolates are non-hemolytic, like *B. anthracis*, and motile, like *B. cereus*.

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- *Chemical Threat Training*
- *Biological Defense Training*

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First described as an agent of anthrax-like disease in gorillas and chimpanzees in Cameroon and Côte d’Ivoire, this organism has since been recovered from an elephant and goats in other countries of Africa. The characteristics of *B. cereus* biovar *anthracis* compared to *B. anthracis* and *B. cereus* are summarized in the following table provided by the Association of Public Health Laboratories (APHL):¹

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

It is worth noting that some *B. cereus* isolates harboring anthrax toxin genes have been reported to cause anthrax-like disease in humans in the United States. There have been a few cases in Texas and Louisiana where metal workers inhaled *B. cereus* G9241 producing a pneumonia like inhalational anthrax. The isolates in these cases do not meet the definition of the *B. cereus* biovar *anthracis*, because they contain only one of the anthrax virulence plasmids; biovar *anthracis* contains both.⁴

In 2013, a patient in Florida developed a facial lesion initially suspected to be an anthrax eschar, which turned out to be caused by a *B. cereus* strain (BcFL2013) like that found in the metal workers.⁵ This case is notable however, because it is the first reported cutaneous anthrax-like eschar caused by *B. cereus*, and the first anthrax-like *B. cereus* to include serological testing. A summary follows; the full article can be accessed at <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0156987>.

The patient, a 70-year-old male with no travel history, was admitted to the hospital with a large, swollen, non-painful lesion resembling an anthrax eschar on his face. The differential diagnosis included anthrax, tularemia, vaccinia, glanders, necrotic

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herpes simplex, brown recluse spider bite, or other cutaneous infection. Culture found a gram-positive, broad rod, that was spore-forming, aerobic, and catalase positive, and therefore genus *Bacillus*, but the Sentinel laboratory ruled out *B. anthracis* as the agent was beta-hemolytic and motile. Despite the laboratory's rule-out, the physician diagnosed the patient with cutaneous anthrax. When the diagnosis of anthrax, a reportable disease, was noted, the Medical Epidemiologist with the Bureau of Epidemiology contacted the Bureau of Public Health Laboratories (BPHL), and BPHL reached out to the Sentinel Laboratory to try to solve the puzzle.

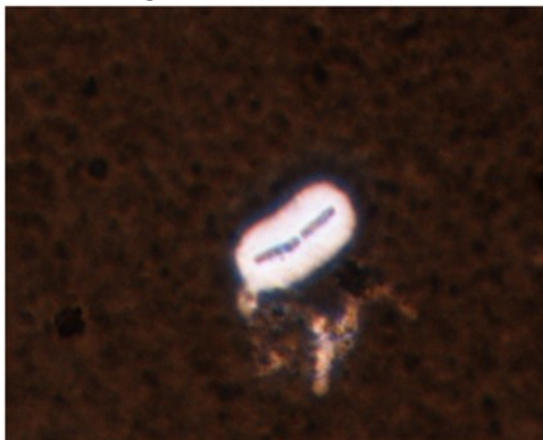
Fig 1. Anthrax-like cutaneous lesion on left check of 70-year-old male Florida resident.



Marston CK, Ibrahim H, Lee P, Churchwell G, Gumke M, et al. (2016) Anthrax Toxin-Expressing *Bacillus cereus* Isolated from an Anthrax-Like Eschar. PLOS ONE 11(6): e0156987. doi:10.1371/journal.pone.0156987 <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0156987>

The Sentinel Laboratory provided a culture of the organism and the original lesion swab to BPHL-Jacksonville, which performed real-time polymerase chain reaction (PCR). One of the three anthrax-specific targets was found (insufficient for an anthrax diagnosis), the target which encodes for anthrax toxin genes. Conventional microbiology confirmed the Sentinel Laboratory's rule-out of *B. anthracis* and identified *B. cereus*. CDC was consulted, and the isolate was shipped to CDC for additional testing and genomic sequencing.

Fig 2. *B. cereus* BcFL2013 capsule. India ink stain of *B. cereus* BcFL2013 after overnight growth in defibrinated horse blood.



Marston CK, Ibrahim H, Lee P, Churchwell G, Gumke M, et al. (2016) Anthrax Toxin-Expressing *Bacillus cereus* Isolated from an Anthrax-Like Eschar. PLOS ONE 11(6): e0156987. doi:10.1371/journal.pone.0156987 <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0156987>

microbiology confirmed the Sentinel Laboratory's rule-out of *B. anthracis* and identified *B. cereus*. CDC was consulted, and the isolate was shipped to CDC for additional testing and genomic sequencing. At CDC, the isolate was again determined to be *B. cereus*, a strain with all three *B. anthracis* toxin genes, but without the anthrax capsule genes, though it was capsule positive. Its multi-locus sequence type was ST78, like the *B. cereus* G9241 that caused anthrax-like pneumonia in the metal workers previously noted. The patient was treated with antibiotics and, unlike the metal workers from Texas, recovered.

As of this writing, *B. cereus* that produces anthrax-like disease, but does not meet the definition of the new biovar, is not considered a select agent. However, CDC/HHS has solicited public comment on whether other virulent *Bacillus* species should be

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regulated, so there may be other related updates to the Select Agents and Toxins list in the future. Your BPHL Biodefense Coordinators and trainers will keep you updated via this newsletter, but don't hesitate to reach out with questions anytime.

Special thanks to Phil Lee, MSc, FIBMS, Lead Biological Defense Coordinator, Bureau of Public Health Laboratories-Jacksonville, for sharing his material regarding the 2013 Florida case.

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References.

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Quick Q&A:

Q. Where can I find an updated Select Agents and Toxins List?

A. <http://www.selectagents.gov/SelectAgentsandToxinsList.html> (under Select Agents and Toxins tab)

*Q. If I suspect my Sentinel Laboratory has a *B. cereus* biovar *anthracis*, or another anthrax-like *B. cereus* strain, what should I do?*

A. Contact your local Bioterrorism coordinators at your nearest public health laboratory—Jacksonville, Tampa, or Miami—as you would for other select agents. A directory can be found on the last page of this newsletter.

Q. Will Sentinel Labs receive rule-out protocols, bench cards, etc. for the new biovar?

A. "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*."¹

*Q. Are there any tests a Sentinel Lab can do to distinguish between *B. cereus* biovar *anthracis* and *B. anthracis*?*

A. "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."¹

BIOSAFETY RISK ASSESSMENT AND LAB BIOSAFETY TRAINING



The Bureau of Public Health Laboratories biosafety outreach officers (BOOs) are currently offering a course in biosafety risk assessment and laboratory biosafety to clinical laboratory institutions. The training consists of two sessions that are approximately one hour each and offered on-site at no charge to the facility. The first session discusses biosafety risk assessment and the second session focuses on biosafety in the clinical laboratory.

Biosafety risk assessment is a systematic process of evaluating the potential risks involved in a laboratory procedure and determining the measures needed to manage any gaps or risks identified. The BOOs have created standard operating procedures and resource documents to assist clinical hospital laboratories in biosafety risk assessment and laboratory biosafety. This session will train clinical laboratory personnel how to use these documents to perform risk assessments in their laboratory.

The second session is for anyone who works in the laboratory or is responsible for a safe working environment. Topics include general laboratory biosafety, the use of biological safety cabinets (BSCs), choosing correct personal protective equipment, proper use and removal of gloves, and spill cleanup. This training awards Florida clinical laboratory and nursing continuing education credits.

For more information or to schedule training, contact the biosafety outreach officer in your region.

For sentinel clinical laboratory locations in Central and South Florida:

Ed Kopp
Office: (813) 233-2260
Cell: (813) 285-1491
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For sentinel clinical laboratories in the Panhandle and North Florida:

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Editor - Betty Wheeler

The CT laboratory coordinators continue to reach out to the health and medical community by offering training for CT preparedness at hospitals and county health departments (CHDs). This training covers chemical terrorism awareness and the collection of clinical specimens after a chemical terrorism event. Hospital and CHD staff play an important role in the response to a chemical exposure event since clinical specimens will be collected for analysis. For your convenience and to increase participation, this training can be presented at your facility. Each course lasts approximately one hour with one 15-minute break between courses. Florida clinical laboratory and nursing continuing education credits will be offered. Training manuals, “hands on” exercise materials, and CT preparedness kits will be provided. This training is recommended for physicians, nurses, epidemiologists, emergency department personnel, phlebotomists, hospital and health department laboratory personnel, and others who may collect clinical specimens. Contact the CT laboratory coordinators in your region for more information (see the Bureau of Public Health Laboratories Directory on the back of this document for contact information).

LABORATORY RESPONSE NETWORK (LRN) TRAINING— BIOLOGICAL DEFENSE

The Bureau of Public Health Laboratories is currently offering an LRN sentinel laboratory training course at no cost to you at your facility. This training follows the American Society for Microbiology (ASM) Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases. Scheduling the training at your facility is a relatively easy process. Determine when you would like to have the training and how many people will be attending. A time will be set up that is convenient for all. The training materials are provided, as well as the biodefense reference manuals for your laboratory.

*“at no cost
to you at
your facility”*

The training syllabus includes: 1) an overview of the LRN; 2) the ASM protocols for ruling out potential bioterrorism agents and how to refer a sample to the state LRN Public Health Reference Laboratory when a bioterrorism agent cannot be ruled out; 3) the role of the sentinel laboratory in responding to pandemic influenza; 4) a brief introduction to packaging and shipping of infectious substances; 5) an introduction to the CDC Select Agent Program; and 6) the College of American Pathologists Laboratory Preparedness Exercise (CAP LPX).

This class awards Florida clinical laboratory continuing education credits based on five hours of instruction. Please contact Betty Wheeler at (904) 791-1568 (Betty.Wheeler@FLhealth.gov) to schedule a class for your facility.

**FLORIDA DEPARTMENT OF HEALTH
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