SENTINEL LEVEL CLINICAL LABORATORY GUIDELINES

FOR

SUSPECTED AGENTS OF BIOTERRORISM

AND

EMERGING INFECTIOUS DISEASES

Bacillus anthracis* and *Bacillus cereus* biovar *anthracis

American Society for Microbiology (ASM)

Revised August 2017

For last revision, see website below

https://www.asm.org/Articles/Policy/Laboratory-Response-Network-LRN-Sentinel-Level-C

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PREANALYTICAL CONSIDERATIONS

I. PRINCIPLE

A. Introduction

Bacillus anthracis, the agent of anthrax, is a zoonotic disease that is transmissible to humans through consumption or handling of contaminated products, is an aerobic, spore-forming, nonmotile, large Gram-positive bacterium (4). *Bacillus cereus* biovar *anthracis* strains were identified in the early 2000's in Cameroon (strains CA) and Côte d'Ivoire (strains CI) (1,17). These strains were recovered from gorillas and chimpanzees with anthrax-like disease. The organism has since been recovered from an elephant and goats in other countries of Africa (1). *B. cereus* biovar *anthracis* CA strains are non-hemolytic, motile, and resistant to penicillin G, while *B. cereus* biovar *anthracis* CI strains are non-hemolytic, motile, and sensitive to pencillin G (17); some *B. cereus* biovar *anthracis* strains may exhibit weak beta-hemolysis upon extended incubation (48 h) and may be more hemolytic when incubated in CO_2 at 48 h (see Table 1). *B. anthracis* and *B. cereus* biovar *anthracis* strains are classified as Tier I, Category A agents because of their suitability for use as biothreat agents in an attack or commission of a biocrime. At this time no human infections caused by *B. cereus* biovar *anthracis* have been described. This procedure describes steps to recognize, presumptively identify, and rule out these organisms from clinical specimens in Sentinel Clinical Laboratories. Such laboratories are defined as those who are certified to perform high complexity testing under the Clinical Laboratory Improvement Amendment of 1988 (CLIA'88) by the Centers for Medicare and Medicaid Services (CMS) for the applicable Microbiology specialty. Laboratory in-house testing includes Gram stains, and at least one of the following: lower respiratory tract, wound, or blood cultures.

Sentinel clinical laboratories are not required to register with the Select Agent Program to conduct diagnostic testing for Select Agents, both Tier I and non-Tier 1. Testing for Select Agents may be performed by laboratories as long as the laboratory follows the policies listed in the reporting section of this document when a Select Agent cannot be ruled out. Consult with your designated LRN Reference Laboratory or refer to the CDC Division of Select Agents and Toxins website at http://www.selectagents.gov for questions.

NOTE: To rule out *B. anthracis* and *B. cereus* biovar *anthracis* requires a combination of morphological assessment including Gram stain characteristics, and three simple, conventional tests. Automated systems and manual multi-test kit identification methods have no place in the identification of this organism due to the danger of misidentification and its close relationship to other species within the *Bacillus* genera.

Table 1. Comparison of *B. anthracis*, *B. cereus*, and *B. cereus* biovar *anthracis* characteristics (adapted from reference 17)

Characteristic	*B. anthracis*	*B. cereus*	*B. cereus* biovar *anthracis* CI[1]	*B. cereus* biovar *anthracis* CA[2]
Hemolysis[3]	-	+	-	-
Motility[4]	-	+	+/-	+/-
Gamma phage susceptibility[5]	+	-	-	-
Penicillin G[6]	S	R	S	R
Capsule	+	Absent in vitro	+	+

1: CI = Côte d'Ivoire strains, from chimpanzees
2: CA = Cameroon strains, from gorillas/chimpanzees
3: Hemolysis: + = beta hemolytic on sheep blood agar; - = non-hemolytic
4: Motility: + = motile; - = non-motile. +/- = *B. cereus* biovar *anthracis* strains are usually motile, including those recovered from gorillas, chimpanzees, and elephants; *B. cereus* biovar *anthracis* goat strains from Democratic Republic of the Congo were non-motile (3).
5: Gamma phage susceptibility: + = susceptible; - = resistant.
6: S= susceptible; R = resistant

B. Geographic distribution

Anthrax is endemic in southern Europe, parts of Africa, Australia, Asia, and North and South America. Its persists in arid deserts of the Middle East, Asia, Africa, Australia and South America with the majority of cases reported from Iran, Turkey, Pakistan, and Sudan. Although relatively rare in the United States, over 500 cases in Texas were reported in White Tailed Deer since 2006. Cases have also been reported in livestock in South Dakota, Nebraska, Arkansas, Mississippi, Louisiana, and California.

Currently, *B. cereus* biovar *anthracis* strains have only been found in certain African countries, including Cameroon and Côte 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.

The use of *B. anthracis* as a bioterrorism agent to inflict disease and death following contact with or inhalation of spores has dominated recorded history for centuries (6, 19). Primarily used by many countries, including the United States, for military purposes in the conduct of biowarfare, it gained notoriety in the commission of biocrimes, determined to be hoaxes, throughout the 1990s. Although the LRN was created in late 1999, its role and responsibilities in

preparing for, and responding to bioterrorism increased dramatically during and after the 2001 outbreak. The lessons learned from this national event demonstrated the serious need for training Sentinel Level Clinical Laboratories and preparing them to play a key role in the LRN.

Three naturally occurring cases of anthrax have been reported within the past five years, one case respectively of gastrointestinal, cutaneous, and inhalational (20, 22, 23). These incidences are examples in which Sentinel Level Clinical Laboratories will be challenged by ensuring that all suspicious *Bacillus* spp., have been ruled out based on the use of the LRN designated tests and algorithm for *B. anthracis* and *B. cereus* biovar *anthracis* discussed in this protocol.

C. Diseases and Clinical Presentation

Anthrax is a zoonotic disease that occurs most frequently in herbivorous animals (e.g., cattle, sheep, and goats), which acquire endospores from contaminated soil. Human disease is less common and results from contact with infected animals or with commercial products derived from them, such as wool and hides. Infection can occur in one of three forms:

1. **Cutaneous**, responsible for >95% of naturally occurring cases, is initiated when the bacterium or spores enter the skin through cuts or abrasions, such as when handling contaminated hides, wool, leather, or hair products (especially goat hair) from infected animals (2, 14, 22). Skin infection begins as a raised itchy bump or papule that resembles an insect bite. Within 1 to 2 days, the bump develops into a fluid-filled vesicle, which ruptures to form a painless ulcer (eschar), usually 1 to 3 cm in diameter with a characteristic black necrotic area in the center. Pronounced edema if often associated with lesions to the releases of edema toxin, a major virulent factor produced by the organism. Lymph glands in the adjacent area may also swell. Approximately 20% of untreated cases of cutaneous anthrax result in death either because the infection becomes systemic or because respiratory distress caused by edema in the cervical and upper thoracic regions. Deaths are rare following appropriate antimicrobial therapy, with lesions becoming sterile within 24 h and resolving several weeks later. There are a few case reports of transmission by insect bites, presumably after the insect fed on an infected carcass (24).

2. **Gastrointestinal** anthrax may occur 1 to 7 days following consumption of contaminated undercooked meat from infected animals and is characterized by acute inflammation of the intestinal tract. Initial signs of nausea, loss of appetite, vomiting, and fever are followed by abdominal pain, vomiting of blood, and severe bloody diarrhea. If not treated, the mortality rate ranges from 25 to 60%. Pharyngeal lesions may also occur from ingestion of contaminated food (20).

3. **Inhalational** anthrax results from the inhalation of *B. anthracis* spores and can occur following an intentional aerosol release as was evident in the 2001 anthrax biocrime. Depending on the quantity of aerosolized spores, the

incubation period ranges from 1 to 6 days. Aerosolization of anthrax spores is regarded as the most likely method to be used in a bioterrorism or biocrime event (5, 9, 10, 15). Though the minimum infectious inhaled dose has not been specifically determined, the U. S. Department of Defense (DoD) estimates that the 50% lethal dose for humans is between 8,000 and 10,000 spores (8). Following inhalation, the spores enter pulmonary macrorophages and are carried to the mediastinal lymph nodes. Germination and vegetative growth result in the production of an antiphagocytic capsule and a toxin comprised of three proteins, edema factor, lethal factor, and protective antigen, which play major roles in the virulence and resultant infectious and clinical manifestation (7). Disease onset is gradual and nonspecific. Fever, malaise, and fatigue may be present initially (prodromal stage) and be accompanied by a nonproductive cough and chest discomfort. These initial symptoms are often followed by a short period of severe respiratory distress with labored breathing (dyspnea), perfuse sweating (diaphoresis), high-pitched whistling respiration (stridor), and cyanosis (bluish skin color). Fatal sepsis with generalized hemorrhage, massive hemorrhagic mediastinitis, and necrosis ensue. Shock and death usually occur within 24 – 36 h following the onset of respiratory distress, and in later stages if therapy is not initiated within 48 h following the onset of symptoms, with mortality reaching 95 – 100% (4, 7, 15, 27). Physical findings are usually nonspecific. The chest X-ray is often pathognomic (disease-specific), revealing a widened mediastinum (not always present) with pleural effusions with infiltrates commonly absent. A single case of inhalational anthrax should alert all healthcare workers to the possibility of a bioterrorism or biothreat event (5). Person-to-person transmission of inhalational anthrax has not been confirmed (3, 9).

D. **CDC CASE DEFINITION** (http://wwwn.cdc.gov/nndss/)

A clinically compatible illness with one of the following:

- Culture and identification of *B. anthracis* from clinical specimens by the LRN
- Demonstration of *B. anthracis* antigens in tissues by immunohistochemical staining using both *B. anthracis* cell wall and capsule monoclonal antibodies
- Evidence of a four-fold rise in antibodies to protective antigen between acute and convalescent sera or a four-fold change in antibodies to protective antigen in paired convalescent sera using CDC quantitative anti-PA immunoglobulin G (IgG) ELISA testing
- Documented anthrax environmental exposure and evidence of *B. anthracis*
- DNA (e.g., by LRN-validated polymerase chain reaction) in clinical specimens collected from a normally sterile site (e.g., blood or CSF) or lesion of other affected tissue (skin, pulmonary, reticuloendothelia, or gastrointestinal).

II. SAFETY CONSIDERATIONS

A. According to the 5th Edition of the BMBL, unless you are working with high concentrations of this organism or performing procedures that produce aerosols, *B. anthracis* can be handled using BSL-2 practices (25). Do not process nonclinical (environmental or animal) specimens in hospital or commercial reference laboratories; restrict processing to human clinical specimens only. Nonclinical specimens should be directed to the designated LRN Reference Laboratory.

B. All patient specimens can be handled using BSL-2 practices. BSL-3 precautions, wearing gloves and gown and working in a certified Class II biosafety cabinet (BSC) are recommended when performing activities having a high potential for aerosol production. Subcultures should be performed in a BSC and plates should be taped/shrink sealed, and incubated in 5 – 10% CO_2. All additional testing should be performed only in the BSC while wearing gloves to prevent acquiring infection through the skin.

C. Decontamination of laboratory surfaces is easily accomplished using a fresh solution of 10% bleach. In addition, pipettes, needles, plastic loops, and microscopic slides should be soaked in 10% bleach or 10 – 30% formalin for 24 h before being autoclaved. Phenolics are not sporocidal at the usual working dilutions (12, 25).

III. MATERIALS

1. Media
    a. Standard liquid blood culturing system with manual or instrument detection
    b. BAP
    c. CHOC
    d. MAC (or EMB)
    e. Semi-solid motility medium

2. Reagents
    a. Appropriate disinfectant such as 10% bleach
    b. Gram stain reagents
    c. Catalase (3% hydrogen peroxide)

3. Equipment and supplies

    a. Biosafety cabinet
    b. Personal Protective Equipment (PPE; gloves, solid front gown)
    c. 35 - 37° C incubator (ambient air, 5 – 10% CO_2)
    d. Light microscope with 100x objective and 10x eyepiece
    e. Microscope slides and cover slips
    f. Pipettes, inoculating loops
    g. Blood culture instrument (optional)

NOTE: Refer to General Introduction and Recommendations section for a listing of biochemical test and associate procedures.

IV. QUALITY CONTROL

Perform quality control of media and reagents according to package inserts, most recent CLSI document M22, and CLIA standards, using positive and negative controls. Do not use *B. anthracis* or *B. cereus* biovar *anthracis* as a control organism due to its infectious nature. Examine culture plates for contamination, poor hemolysis, cracks, and drying. Quality control of biochemical tests using a positive and negative reacting organism should generally be performed on each lot of reagent. For frequency of quality control, refer to manufacturer guidelines and state and federal regulation. Refer to biochemical test section (located in the General Introduction and Recommendations Section) of procedures and quality control organisms for each test.

V. SPECIMEN COLLECTION

A. Collection and Transport of Clinical Specimens for Laboratory Rule-Out Testing*	
Cutaneous Vesicular (early) stage Eschar (late) stage	• Unroof vesicle and aspirate fluid or collect with two sterile swabs (dacron) • Insert swab (dacron) beneath the edge of the eschar, rotate swab or obtain an aspirate • Transport specimens at room temperature
Gastrointestinal	• Stool (> 5 grams…pecan size), collect and transport in a leak proof sealed container • Collect blood (late stage of infection) directly into an appropriate blood culture bottle (aerobic and anaerobic) • Transport specimens and bottles at room temperature
Inhalational	• Sputum • Blood: collect directly into an appropriate blood culture bottle (aerobic and anaerobic) • Cerebral Spinal Fluid only if signs of meningitis occur • Transport specimens and bottles at room temperature
Postmortem Tissue	• Tissue pieces should be collected and kept moist • Transport in sterile container at room temperature within 1 hour of collection

References (9, 13, 16, 18, 26)
* additional specimens that may be requested by your designated LRN Reference Laboratory are: plasma, pleural fluid, serum

A. Rejection of specimens
   1. Use established laboratory criteria for rejection of specimens

2. Environmental or non-clinical specimens are not to be processed by Sentinel Laboratories; contact your designated LRN Reference Laboratory directly.

ANALYTICAL CONSIDERATIONS

VI. SPECIMEN PROCESSING

A. Cutaneous
   1. Plate cutaneous, tissue, and stool specimens onto BAP and MAC (or EMB). With the exception of stool specimens, prepare smears for Gram stain.

B. Respiratory
   1. Plate respiratory specimens onto BAP, CHOC, and MAC (or EMB). Prepare smear for Gram stain.

C. Blood
   1. Aseptically inoculate liquid blood culture bottles with appropriate blood volume per manufacturer's instructions.

D. Cerebrospinal fluid (CSF)
   1. Prepare a cytospin smear for Gram stain
   2. Centrifuge CSF specimens at 1,500 x g for 15 min using a clinical centrifuge equipped with appropriate biocontainment tube holders. Open the biocontainment tube holders only in a BSC. Plate the resultant sediment onto BAP and CHOC; aspirate the remaining sediment and inoculate TSB or equivalent.

VII. INCUBATION AND EXAMINATION OF CULTURES

A. Incubate all plated specimens, except MAC (or EMB), at 35 to 37°C in 5-10% CO_2; MAC (or EMB) is incubated in ambient air at 35 to 37°C.
B. Examine all cultures within 18-24 h of incubation. Growth of *B. anthracis* or *B. cereus* biovar *anthracis* may be observed as early as 8 h following incubation. This can be helpful when looking for *B. anthracis* or *B. cereus* biovar *anthracis* from mixed cultures (sputum, stool).

VIII. CULTURE IDENTIFICATION

If your laboratory uses matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry for bacterial identification and if the manufacturer provides your facility with an alternate tube extraction method, it is recommended that the resulting extract be filtered using a 0.2 µ (or less) filter. This additional step is recommended to reduce the risk of laboratory contamination with viable bacteria and spores.

Using automated systems, including MALDI-TOF technology, may result in exposure to dangerous pathogens, and could result in erroneous identification, Ex: *Bacillus anthracis* misidentified as *B. cereus*; *Yersinia pestis* misidentified as *Y. pseudotuberculosis*.

The rule out or referral of *B. anthracis* or *B. cereus* biovar *anthracis* is accomplished by following the algorithm below and the associated tests.

A. Gram stain suspicious colonies from agar plates and blood cultures in a BSC. *B. anthracis* and *B. cereus* biovar *anthracis* are large (1 to 1.5 by 3 to 5um) Gram-positive rods. Vegetative cells seen on Gram-stained smears of clinical specimens often occur in short chains of two to four cells that are encapsulated. Gram stains from colonies grown on BAP appear as long chains of nonencapsulated Gram-positive bacilli. Endospores are not commonly seen in direct smears of clinical specimens. If present, the spores are oval and located centrally or subterminally and do not cause swelling of the vegetative cell (see Fig 1).

Fig. 1. Gram stain of blood culture containing *B. anthracis*

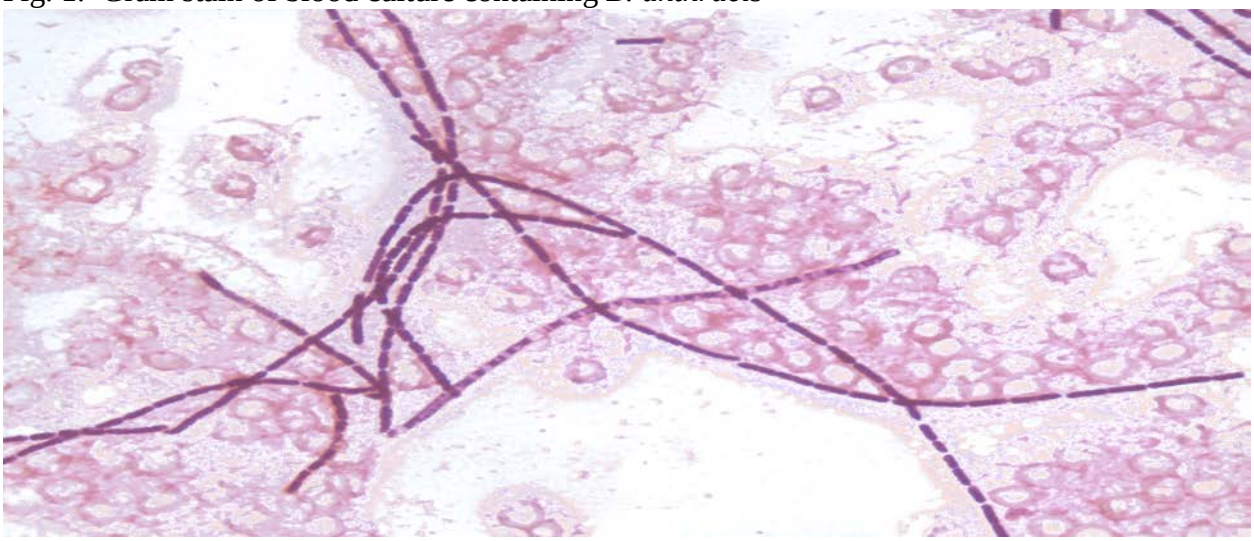


Photo courtesy of Dr. James Rudrick, Michigan Department of Community Health

B. Both *B. anthracis* and *B. cereus* biovar *anthracis* grow well on BAP and CHOC, but not on MAC (or EMB). 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. Some *B. cereus* biovar *anthracis* strains may exhibit weak hemolysis upon extended incubation (48 h), particularly when incubated in CO_2.

Fig. 2. Colonies of *B. anthracis* on Blood Agar

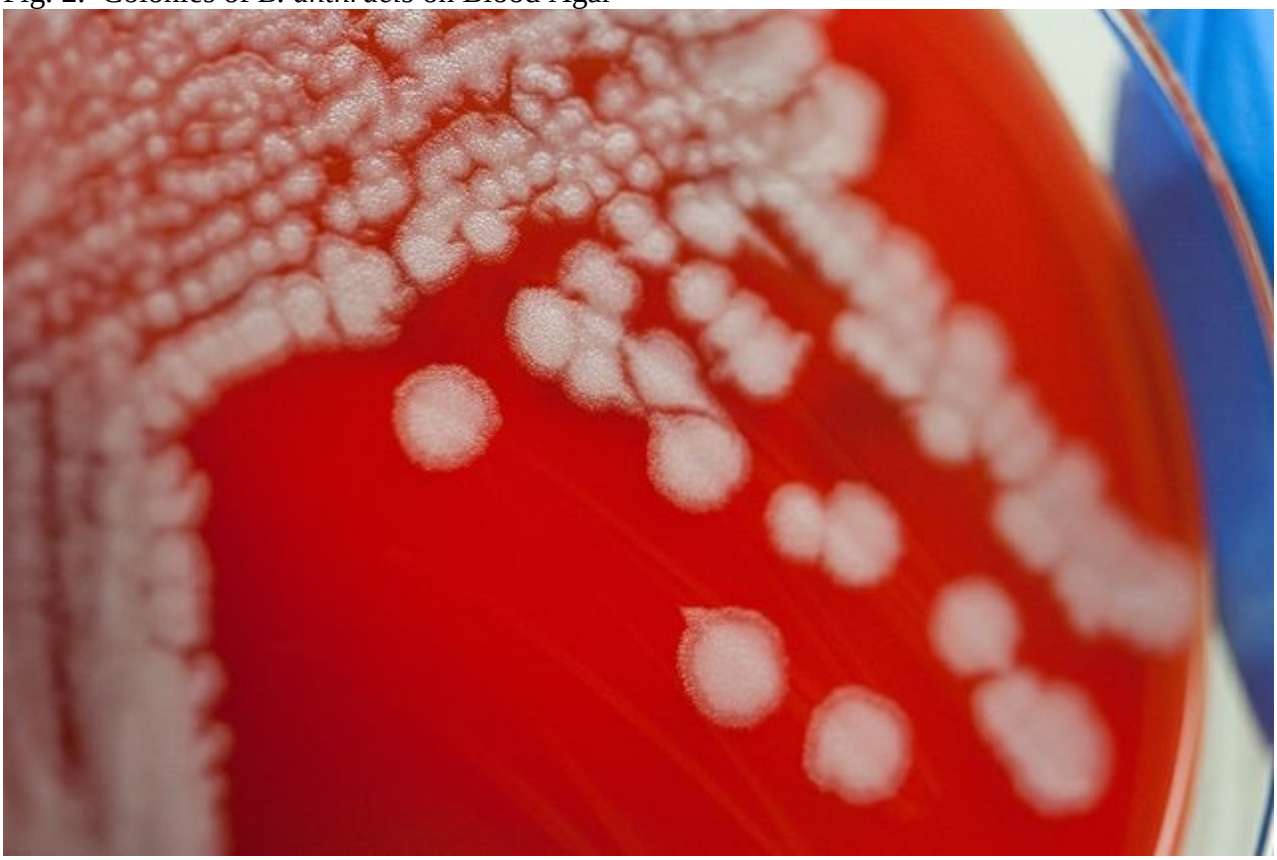


Photo courtesy of APHL

C. Perform all testing in a BSC. Refer to the Biochemical Test Procedures listed in the Introduction and General Recommendations Section.
    1. Catalase – should be positive
    2. Motility in semi-solid medium– *B. anthraci*s is usually negative; *B. cereus* biovar *anthracis* strains are usually motile (Table 1).

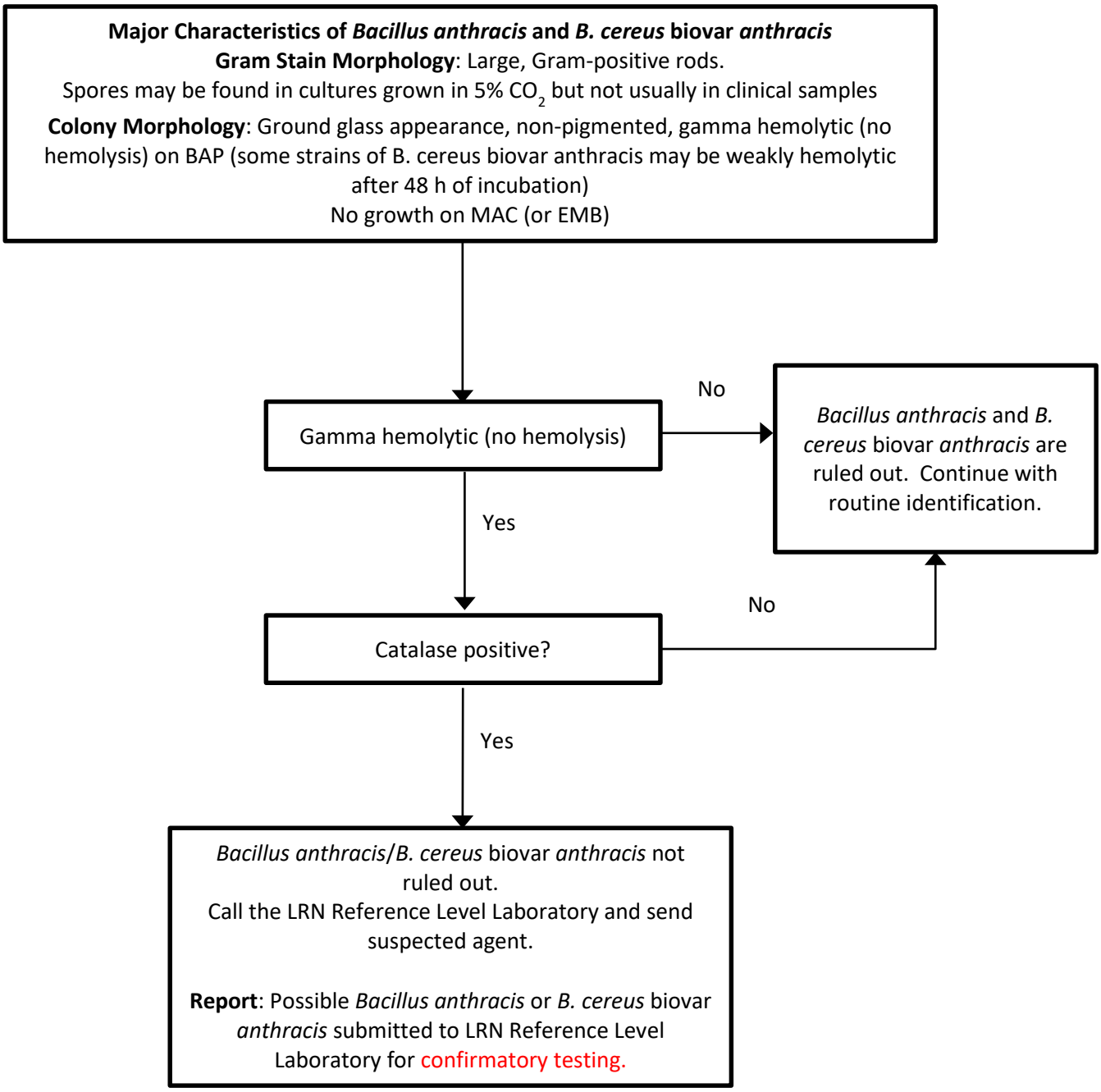
NOTE: Performing a catalase test is potentially hazardous due to the potential for the creation of an aerosol thus it is recommended to perform this test in a BSC. It is recommended to perform motility in semi-solid medium instead of a wet mount since results are less subjective in semi-solid medium.

D. Presumptive identification of *B. anthracis* and *B. cereus* biovar *anthracis* (see flow chart below)

NOTE: Confirmatory identification is made by an LRN Reference Laboratory; refer to http://www.bt.cdc.gov/lrn/biological.asp

1. Direct Gram stain: large, Gram-positive rods; Spores are not normally observed in smears from clinical specimens.
2. Colonies on BAP are nonhemolytic, project a ground glass appearance, and may have “Medusa head” characteristics. If endospores are present, they are oval-shaped and located either centrally or subterminally.

Bacillus anthracis and *B. cereus* biovar *anthracis* Identification Flowchart



Note: Biochemical test procedures and quality control instructions can be found at the end of the General Recommendation and Biochemical Testing Procedures document.

POST ANALYTICAL CONSIDERATIONS

IX. REPORTING, NOTIFICATION, AND TRANSFER

A. *B. anthracis* or *B. cereus* biovar *anthracis* is suspected and cannot be ruled out if the isolate fulfills the following characteristics:
- Direct Gram staining reveals large Gram-positive rods
- Grows on BAP as non-pigmented, odorless, white colonies having a ground glass appearance with edges that are slightly undulate ("Medusa heads"; comma-shaped)
- Does not grow on MAC (or EMB)
- Nonhemolytic on BAP
- Catalase – positive
- Motility – *B. anthracis* strains are nonmotile, *B. cereus* biovar *anthracis* strains are motile

B. Notification and submission of cultures if *B. anthracis* or *B. cereus* biovar *anthracis* cannot be ruled out based on the above characteristics.
1. Generate a report to the attending physician that *B. anthracis* or *B. cereus* biovar *anthracis* cannot be ruled out and the isolate has been referred to your designated LRN Reference Laboratory for confirmatory identification.
2. Do not attempt full identification and susceptibility testing in the Sentinel Level Clinical Laboratory.
3. Immediately notify your designated LRN Reference Laboratory who will provide the referring laboratory with guidance and recommendations for retaining the specimen or isolate and submission for confirmatory identification.
4. Preserve original specimens pursuant to a criminal investigation and transfer to your designated LRN Reference Laboratory in accordance with state and local requirements. In particular, the appropriate material, including blood culture bottles, tubes, plates, and clinical specimens (e.g., aspirates, biopsies, sputum specimens) should be documented and either submitted to the LRN Reference Laboratory or retained until the Reference Laboratory confirms the identification.
5. Do not ship specimens or cultures to the LRN Reference Laboratory without prior arrangements.
6. Notify other public health authorities (e.g., state public health department Epidemiologist/Health Officer) as required by local and state communicable disease reporting requirements. The state public health laboratory/department will notify law enforcement officials (state and federal), such as the local FBI, as appropriate.
7. Within the hospital setting, immediately notify the infection control preventionist(s) and/or infectious disease service so that the patient can be treated appropriately, infectious precautions can be taken, and a further investigation of the patient's history can be made (10).

8. Consult with the LRN Reference Laboratory regarding additional clinical specimens that may be submitted for testing.
9. Initiate documentation showing the specimen identification control, notification and transfer to your designated LRN Reference Laboratory, and documentation of all plates and tube cultures which will need to be destroyed or transferred once identification has been completed.

C. Sentinel Level Clinical Laboratories should consult with their designated LRN Reference Laboratory prior to or concurrent with testing if *B. anthracis* or *B. cereus* biovar *anthracis* is requested by the physician or a bioterrorist event is suspected. Obtain guidance from the state public health laboratory as appropriate (e.g., requests from local law enforcement or other local government officials). The FBI and state public health laboratory/state public health department will coordinate the transfer of isolates/specimens to a higher level LRN laboratory as appropriate.

D. If *B. anthracis* or *B. cereus* biovar *anthracis* is ruled out, proceed with efforts to identify using established criteria.

E. If other cases are suspected or there is a laboratory exposure, collect appropriate samples and submit them to your designated LRN Reference Laboratory for additional testing.

X. Select Agent reporting and compliance

1. Reporting of all identified Select Agents is still required, even though Sentinel laboratories are not required to register under the Select Agent Rule.
2. The laboratory must complete Form 2 within one week (7 days) following notification of the confirmed identification. For further guidance and access to the necessary forms, consult with your designated LRN Reference Laboratory or refer to the CDC Division of Select Agents and Toxins website at www.selectagents.gov
3. Reporting all identified Select Agents is required by completing Form 4 A within 7 days of confirmed identification. If the isolate is from a Proficiency test sample, Form 4 B is to be completed within 90 days of receipt of the sample.
4. Your designated LRN Reference Laboratory will advise you with completion of required forms (e.g., Forms 2, 3, and 4). Always refer to www.selectagents.gov for the latest guidance and versions of these forms.

XI. SUMMARY/SPECIAL CONSIDERATIONS

A. Antimicrobial Susceptibility

1. Antimicrobial susceptibility testing of *B. anthracis* or *B. cereus* biovar *anthracis* is neither needed nor appropriate for Sentinel Level Laboratories to perform. Interpretive breakpoints have not been established by the CLSI.
2. Quinolones (e.g. ciprofloxacin, levofloxacin), doxycline, and penicillin are currently the only FDA-approved antibiotics for the treatment of anthrax (2, Center for Biosecurity of UPMC, www.upmc-biosecurity.org).
3. Post-exposure prophylaxis for personnel (adults and children), including laboratory personnel suspected of exposure to *B. anthracis* or *B. cereus* biovar *anthracis* spores includes ciprofloxacin, levofloxacin (adults) or doxycline. Once started, antibiotic therapy should be continued for 60 days post-exposure (www.upmc-biosecurity.org).

B. Select Agent Reporting and Compliance

1. Reporting of all identified Select Agents is still required, even if a laboratory has not been previously registered.
2. If the organism is transferred following presumptive identification, the laboratory must complete Form 2. For further guidance and access to the necessary forms, consult with your designated LRN Reference Laboratory or refer to the CDC Division of Select Agents and Toxins website: http://www.selectagents.gov
3. Reporting all identified Select Agents is required by completing Form 4A within 7 days of confirmatory identification. If the isolate is from a Proficiency Test Sample, Form 4B is to be completed within 90 days of receipt of the sample.
4. Your designated LRN Reference Laboratory will advise you with completion of required forms (e.g. Forms 2, 3, and 4). Always refer to www.selectagents.gov for the latest guidance and versions of these forms.

C. Destruction

1. Once the identification of the isolate has been confirmed, the Sentinel Clinical Laboratory Select Agent Regulations require that the residual specimen and cultures of the isolate be destroyed or transferred to an approved Select Agent entity within 7 days of notification of a confirmed identification. Your designated LRN Reference Laboratory must advise you on destruction or transfer of isolates.
2. Generally, all plates, tubes, and clinical material that contain the organism should be autoclaved, incinerated on-site or submitted to the designated LRN Reference Laboratory for disposal.
3. Alternatively, contaminated items should be soaked in 10% bleach or 10% formalin for 24h.

D. Packing and Shipping

1. Refer to the ASM Packing and Shipping Guidelines
2. All materials sent to your designated LRN Reference Laboratory must be shipped in compliance with IATA and DOT regulations.

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