



REPLY TO
ATTENTION OF

MCHO-CL

DEPARTMENT OF THE ARMY
HEADQUARTERS, U S ARMY MEDICAL COMMAND
2050 WORTH ROAD
FORT SAM HOUSTON, TEXAS 78234-6000



16 OCT 2001

MEMORANDUM FOR COMMANDERS, ARMY MEDICAL TREATMENT FACILITIES

SUBJECT: Management of Patients with Potential Exposure to Anthrax Spores

1. References.

a. Information Paper, DASG-HSZ, 16 October 2001, SUBJECT: Clinical Management of Patients Potentially Exposed To Anthrax Spores (enclosed).

b. US Army Medical Research Institute of Infectious Diseases. Medical Management of Biological Casualties, 4th ed. 2001 Feb
(<http://www.usamriid.army.mil/education/bluebook.html>)

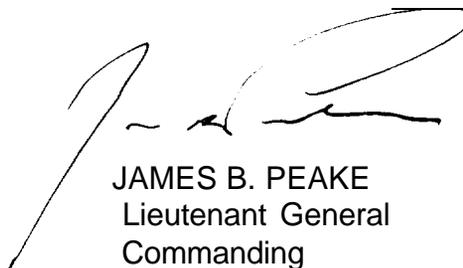
c. Centers for Disease Control and Prevention. CDC Guidelines for State Health Departments - Responding to "Anthrax Threats" and Anthrax (B. anthracis) Diagnostic Testing. Revised October 14, 2001.(enclosed)

d. Standard Operating Procedure, the presumptive identification of the Bacillus anthracis. (enclosed)

2. Enclosed guidance (ref 1.a.) provides a standard AMEDD clinical approach to the patient possibly exposed to anthrax. It is my intent that all Military Health System beneficiaries with complaints of potential exposure to anthrax be evaluated according to these guidelines.

3. My point of contact for clinical issues relating to potential anthrax exposures is LTC Scott Stanek , Operational Medicine Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, DSN 343-4996, commercial301-619-4996, email Scott.Stanek@amedd.army.mil. The point of contact for laboratory issues is COL James C. Bolton, DSN 471-6344, Commercial (210) 221-6344 or email James.Bolton@cen.amedd.army.mil.

3 Enclosures



JAMES B. PEAKE
Lieutenant General
Commanding

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Commander, US Army Center for Health Promotion and Preventive Medicine

Commander, Medical Research and Materiel Command

Commander, US Army Research Institute for Infectious Diseases

INFORMATION PAPER

SUBJECT: Clinical Management of Patients Potentially Exposed To Anthrax Spores

1. Purpose. To describe standardized clinical procedures and laboratory evaluation of patients presenting to AMEDD Military Treatment Facilities (MTFs) with potential exposure to anthrax spores.

2. References.

a. US Army Medical Research Institute of Infectious Diseases. Medical Management of Biological Casualties, 4th ed. 2001 Feb (<http://www.usamriid.army.mil/education/bluebook.html>)

b. Centers for Disease Control and Prevention. CDC Guidelines for State Health Departments - Responding to "Anthrax Threats" and Anthrax (B. anthracis) Diagnostic Testing. Revised October 14, 2001.

c. Centers for Disease Control and Prevention. How to handle anthrax and other biological agent threats. October 12, 2001. See Resources: Agents/Diseases -- Bacillus anthracis link (<http://www.bt.cdc.gov>).

d. Centers for Disease Control and Prevention. Basic laboratory procedures for the presumptive identification of Bacillus anthracis. April 18, 2001. See Resources: Agents/Diseases -- Bacillus anthracis links at (<http://www.bt.cdc.gov>).

e. Centers for Disease Control and Prevention. Use of Anthrax Vaccine in the United States. Morbidity and Mortality Weekly Report, Vol. 49, No. RR-15 15 Dec 00.

f. Inglesby TF, Henderson DA, Bartlett JG, et al. Anthrax as a biological weapon: medical and public health management. *JAMA* 1999;281:1735-1745.

3. Facts.

a. Clinical Presentation. Anthrax is an illness with acute onset characterized by several distinct clinical forms, including the following:

- Cutaneous: a skin lesion evolving during a period of 2-6 days from a papule, through a vesicular stage, to a depressed black eschar
- Inhalation: a brief prodrome resembling a viral respiratory illness, followed by development of hypoxia and dyspnea, with radiographic evidence of mediastinal widening

- Intestinal: severe abdominal distress followed by fever and signs of septicemia
- Oropharyngeal: mucosal lesion in the oral cavity or oropharynx, cervical adenopathy and edema, and fever

b. **Medical Surveillance and Clinical Suspicion.** Health care providers play a key role in identifying and reporting illnesses that result from acts of bioterrorism. In support of this role, all providers must increase their level of diagnostic suspicion for illnesses resulting from infection with *Bacillus anthracis*, even in the absence of any specific history of potential exposure to anthrax spores. Health-care providers will watch for potential cases of maliciously induced disease in the course of routine health care. Also, to enhance the ability of the Army's automated surveillance system, named ESSENCE, to identify clusters of illnesses potentially caused by *B. anthracis*, providers and other support personnel must submit and process information through the Ambulatory Data System (ADS) on all patient visits in a timely manner (before close of business each day).

c. **Patient Assessment.** MTFs will train personnel to evaluate asymptomatic and symptomatic patients potentially exposed to anthrax spores in the following way (see attached diagram):

(1) Asymptomatic patients with potential exposure to anthrax. If a patient claims a potential anthrax exposure, a history (to include an assessment of the incident) and examination will be performed to determine the level of the credibility for this claim. The assessment of the incident should include discussions with local law enforcement officials. A physical examination will be performed, including a careful dermatological exam to identify cutaneous lesions that appear at 2-7 days after exposure. Factors to be taken into consideration in determining whether a credible exposure has occurred include the occurrence of other locally, confirmed cases, the existence of a powder or other source material which possibly contains anthrax spores, or other situational factors indicating a possible bioterrorist attack. If in the judgment of the attending physician, a credible anthrax exposure has occurred, the following clinical specimen should be collected:

Nasal swabs for culture and gram stain
(Use a Rayon swab moistened with sterile distilled water or saline)

(2) Symptomatic patients with a presumed diagnosis of anthrax disease. If a health care provider makes a tentative diagnosis of anthrax based on history and clinical presentation, the following clinical specimens will be obtained:

- (a) For all patients
- Nasal swabs for culture and gram stain
(Use a Rayon swab moistened with sterile distilled water or saline)
 - Blood culture

(b) Cutaneous anthrax

- Vesicular stage: The organism is best demonstrated in this stage. Soak two dry sterile swabs in vesicular fluid from a previously unopened vesicle.
- Eschar stage: Rotate two swabs beneath the edge of the eschar without removing the eschar.

(c) Gastrointestinal anthrax

- If the patient is able to produce a stool specimen, stool cultures should be performed. Obtain a stool culture.
- In later stages of disease, blood cultures will yield the organism, especially if specimens are obtained prior to antibiotic treatment.

(d) Inhalation anthrax

- If respiratory symptoms are present and sputum is being produced, obtain a specimen for culture and smear.
- In later stages of disease (2-8 days post exposure) blood cultures may yield the organism, especially if specimens are drawn before antibiotic treatment. In addition, a biopsy of any suspicious cutaneous lesions should be taken from the border area to include an erythematous edge, if possible.

d. Laboratory Evaluation. MTF laboratories will adhere to previously published standard operating procedures (attached) for evaluation of specimens collected from patients suspected to be infected with anthrax. Laboratories should also note that:

(1) Some threat materials are very prone to aerosolization. After processing threat letters and samples, one network laboratory had to be closed for several days and required extensive decontamination.

(2) Threat powders may appear slightly gray, off white, or light beige. If the threat material is crystalline (like sugar, Epsom salt or table salt), it is unlikely that a biological threat exists - but the material may be a chemical threat. Plain letters without any powder or other materials rarely pose an immediate threat.

(3) If you must handle threat materials, ensure that you gown, wear gloves, and mask. Materials should only be opened in a biohazard hood - never in an open laboratory or on a bench top.

(4) Threat materials containing powders should be double- or triple-bagged. Local law enforcement authorities should be notified. Do not ship materials to other network laboratories unless instructed to do so. Shipment of threat materials must be closely coordinated with destination laboratories. Until shipment can be arranged - place threat materials in a cool, dry and secure place under lock and key. Inventory and account for materials.

e. Post-exposure prophylaxis for asymptomatic patients. When in the judgment of the attending physician a credible exposure to anthrax has occurred, ciprofloxacin

(500 mg p.o. bid for adults) or doxycycline (100 mg p.o. bid for adults) should be administered. If susceptibility testing demonstrates susceptibility to amoxicillin, therapy should be changed to oral amoxicillin (500mg p.o. q 8 hours for adults) to complete the 60 day course of prophylaxis. In the event that a source material which was originally suspected to possibly contain anthrax spores is confirmed to not have anthrax, the exposure may be reassessed as non-credible and prophylaxis may be discontinued. For patients with credible exposures who complete a full 60-day course of prophylaxis, they should be subsequently closely observed. If clinical signs of anthrax occur, empiric therapy for anthrax is indicated, pending etiologic diagnosis. For more information on the use of prophylactic medications in adults, pregnant women, and children, see guidance from Centers for Disease Control, reference 2.b.

f. Presumptive Diagnosis.

(1) Signs and Symptoms. The incubation period for inhalational anthrax is 1-6 days. Fever, malaise, fatigue, cough, and mild chest discomfort are rapidly followed by severe respiratory distress with dyspnea, diaphoresis, stridor, and cyanosis. Shock and death occur within 24-36 hours after the onset of severe symptoms. In cases of cutaneous anthrax, a papule develops, then vesiculates, finally developing into a black eschar surrounded by moderate edema. The lesions are usually painless.

(2) Physical Findings. Physical findings are typically non-specific in inhalational cases, with initial complaints of malaise, fever, headache, and possibly substernal chest pain. A widened mediastinum is sometimes seen on x-ray late in the course of illness, and correlates with a pathologic finding of hemorrhagic mediastinitis, the "classic" presentation of inhalational anthrax.

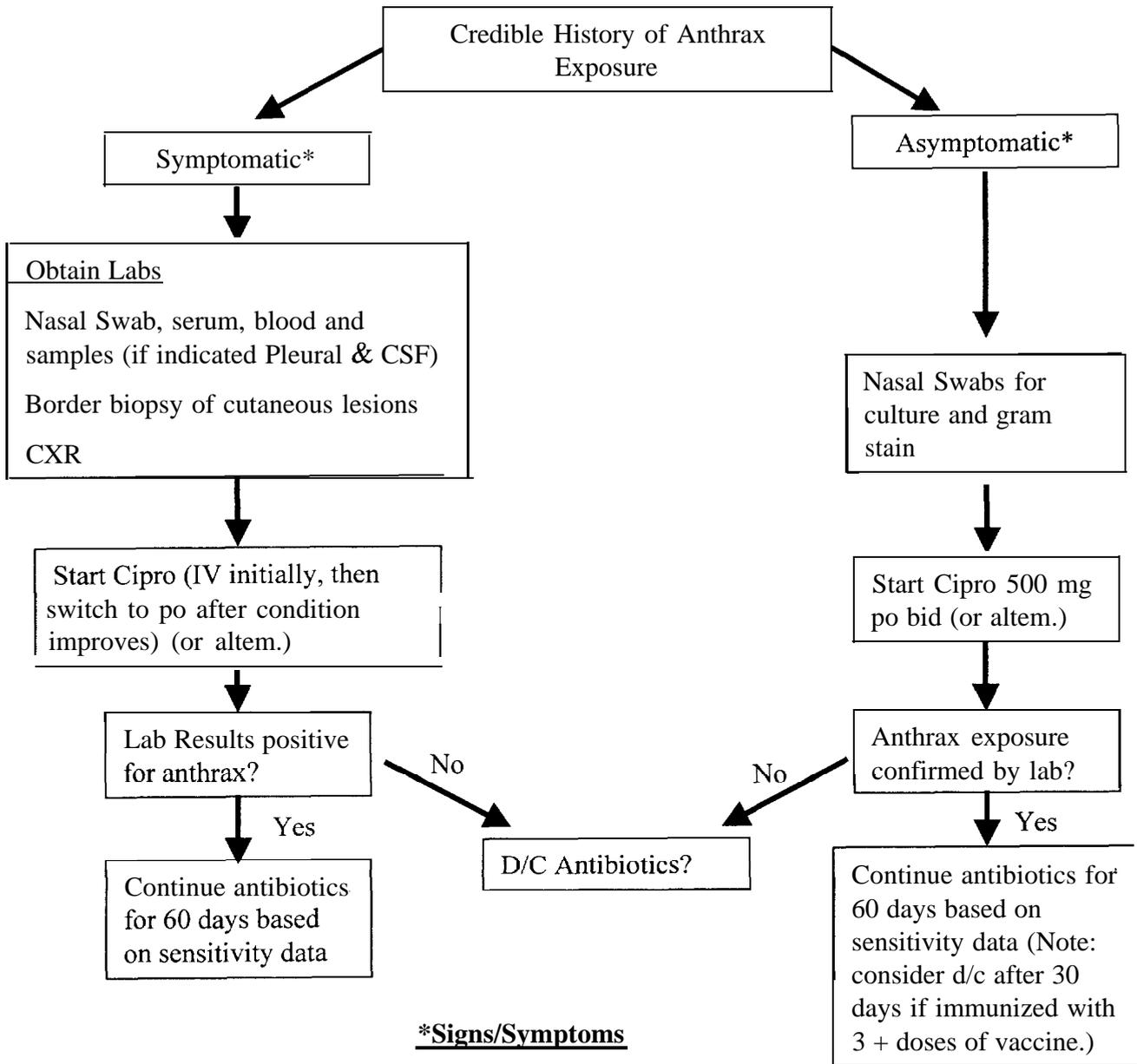
g. Definitive Treatment. In the absence of antibiotic sensitivity data, empiric intravenous antibiotic treatment should be instituted at the earliest signs of disease. Military policy (FM 8-284) currently recommends ciprofloxacin (400 mg IV q 12 hrs) or doxycycline (200 mg IV load, followed by 100 mg IV q 12 hrs) as initial therapy, with penicillin (4 million U IV q 4 hours) as an alternative once sensitivity data is available. Published recommendations from a public health consensus panel recommends ciprofloxacin as initial therapy. Therapy may be tailored once antibiotic sensitivity of the bacterial isolate is available. Recommended treatment duration is 60 days, and should be changed to oral therapy as clinical condition improves. Supportive therapy for shock, fluid volume deficit, and adequacy of airway may all be needed.

h. Patient Isolation Precautions. Anthrax cannot be transmitted from person to person. Standard infection control precautions are sufficient for patients diagnosed with anthrax or have a known or suspected exposure to anthrax. Patient isolation or quarantine is not required. Note: If it is suspected that the patient's clothing may be contaminated, the clothing should be placed in a plastic bag and given to law enforcement officials.

i. Decontamination. Dermal exposure to a suspected anthrax aerosol should be immediately treated by soap and water decontamination. Careful washing with soap and water removes nearly all of the agent from the skin surface. Hypochlorite solution (bleach) or other disinfectants are reserved for gross contamination (i.e. following the spill of solid or liquid agent from a munition directly onto the skin). In the absence of chemical or gross biological contamination, these will confer no additional benefit, may be caustic, and may predispose to colonization and resistant superinfection by reducing the normal skin flora. Grossly contaminated skin surfaces should be washed with a 0.5% sodium hypochlorite solution, if available, with a contact time of 10 to 15 minutes. Following invasive procedures or autopsy, instruments and surfaces should be thoroughly disinfected with a sporicidal agent (high-level disinfectants such as iodine or 0.5% sodium hypochlorite.)

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Anthrax Exposure Algorithm



***Signs/Symptoms**

Incubation period: 1-6 days, may be longer in some cases

Early s/s: Fever, malaise, fatigue, cough, mild chest discomfort, unusual dermal lesion

Late s/s: resp. distress, dyspnea, diaphoresis, stridor, cyanosis

CDC Guidelines for State Health Departments Revised October 14, 2001

I. Responding to “Anthrax Threats” and Anthrax (*B. anthracis*) Diagnostic Testing

Anthrax Threats (Letters, packages, etc.)

[Instructions for individuals are located in the Official CDC Health Alert sent on October 12, 2001: “HOW TO HANDLE ANTHRAX AND OTHER BIOLOGICAL AGENT THREATS”]

Dealing with a suspicious package

- Do not open the letter.
- If the letter has already been opened and **powder spills out, do not clean it up. Keep others away from the area.**
- Double bag the letter; plastic is best (use plastic/rubber gloves and a particulate mask if available).
- Immediately wash your hands with soap and water.
- Notify your supervisor, law enforcement officials, and the FBI
- Notify local, county, and state health officials
- Evacuate the area
- Ensure that all persons who have handled the letter wash their hands
- Start a list of names and telephone numbers all persons who have handled the letter
- Give potentially exposed persons information about the signs and symptoms of illness associated with the biologic agent and about whom to contact and where to go should they develop illness.
- Place all clothing items worn when in contact with the letter into plastic bags
- Keep these bags with you, so that they are available for law enforcement officials
- As soon as possible shower with soap and water

Asymptomatic patient WITHOUT known exposure

- Provide reassurance to the patient about the rarity of infection without known exposure.
- Recommend the patient see a health care provider for further concerns and/or diagnostic tests.
- It's important for people to know that there is no screening test indicated for the detection of anthrax infection in an asymptomatic person. Nasal swabs should not be used for diagnosis. Nasal swabs and blood serum tests are used as an epidemiological tool to characterize an outbreak when there is a known biologic agent.

Asymptomatic patient WITH known exposure

- Conduct an individual risk assessment with public health officials and refer to a health care provider if post-exposure prophylaxis is necessary. Rapid screening assays, which can be performed directly on clinical specimens and environmental samples, are being made available for restricted use in Laboratory Response Network “B” and “C” level laboratories.

- In this circumstance, decontaminating the patient, other than by washing with soap and water, is not routinely recommended.
- In Florida, susceptibility testing determined that the isolate was penicillin susceptible, and therefore amoxicillin was indicated as a first-line agent.
- Post-exposure Prophylaxis (PEP) Recommendations

	Initial therapy	Duration
Adults (including pregnant women ^{1,2} and immunocompromised)	Ciprofloxacin 500 mg po BID Or Doxycycline 100 mg po BID	60 days
Children ^{1,3}	Ciprofloxacin 15-20 mg/kg po Q12 hrs ⁴ Or Doxycycline ⁵ : >8 yrs and >45 kg: 100 mg po BID >8 yrs and ≤ 45 kg: 2.2 mg/kg po BID ≤ 8 yrs: 2.2 mg/kg po BID	60 days

1. If susceptibility testing allows, therapy should be changed to oral amoxicillin for post-exposure prophylaxis to continue therapy out to 60 days.
2. Although tetracyclines are not recommended during pregnancy, their use may be indicated for life-threatening illness. Adverse affects on developing teeth and bones are dose related, therefore, doxycycline might be used for a short course of therapy (7-14 days) prior to the 6th month of gestation. Please consult physician after the 6th month of gestation for recommendations.
3. Use of tetracyclines and fluoroquinolones in children has adverse effects. These risks must be weighed carefully against the risk for developing life-threatening disease. If a release of *B. anthracis* is confirmed, children should be treated initially with ciprofloxacin or doxycycline as prophylaxis but therapy should be changed to oral amoxicillin 80 mg/kg of body mass per day divided every 8 hours (not to exceed 500 mg three times daily) as soon as penicillin susceptibility of the organism has been confirmed.
4. Ciprofloxacin dose should not exceed 1 gram/day in children.
5. In 1991, the American Academy of Pediatrics amended their recommendation to allow treatment of young children with tetracyclines for serious infections, such as, Rocky Mountain Spotted Fever, for which doxycycline may be indicated. Doxycycline is preferred for its twice-a-day dosing and low incidence of gastrointestinal side effects.

Patients with Symptoms Compatible with Anthrax

- Notify local and state public health officials so they can begin an epidemiologic investigation.
- Confirm the diagnosis by obtaining the appropriate laboratory specimens based on the clinical form of anthrax that is suspected (inhalational, gastrointestinal, or cutaneous).
 - Inhalational anthrax: blood, CSF (if meningeal signs are present); chest X-ray
 - Gastrointestinal anthrax: blood
 - Cutaneous anthrax: vesicular fluid and blood

Evaluation of possible anthrax infection for individuals not connected with the AMI incident in Florida should be performed through standard laboratory tests, following the Laboratory Response Network (LRN¹) Level A Clinical Guidelines for rule out and presumptive testing <http://www.bt.cdc.gov> (follow the link for Resources: Agents/Diseases -*Bacillus anthracis*)

- a. Presumptive identification criteria (level A LRN laboratory)
 1. From clinical samples, such as blood, CSF, or skin lesion (vesicular fluid or eschar) material: encapsulated Gram-positive rods
 2. From growth on sheep blood agar: large Gram-positive rods
 3. Non-motile
 4. Non-hemolytic on sheep blood agar

Additional LRN level B laboratory criteria for confirmation of *B. anthracis* are available through State Public Health Laboratories and involve:

- b. Confirmatory criteria for identification of *B. anthracis* (level B LRN laboratory)
 1. Capsule production (visualization of capsule), and
 2. Lysis by gamma-phage, or
 3. Direct fluorescent antibody assays (DFA)

Rapid screening assays, such as nucleic acid signatures and antigen detection, which can be performed directly on clinical specimens and environmental samples, are being made available for restricted use in LRN B and C level laboratories.

¹ *Laboratory Response Network for Bioterrorism* (LRN) is a collaborative partnership and multilevel system designed to link state and local public health laboratories with advanced capacity clinical, military, veterinary, agricultural, water and food-testing laboratories. The LRN operates as a network of laboratories (laboratory levels designated A: hospital laboratories, B: state health laboratories, C: CDC laboratory, D: CDC and USAMRIID) with progressively stringent levels of safety, containment and technical proficiency necessary to perform the essential rule-out, rule-in, and referral functions required for agent identification. Network access provides all public health laboratories with the means to accept and transfer specimens to appropriate facilities where definitive testing can be undertaken. This facilitates early detection and **suspect-level** identification at the local clinical laboratory level, which is subsequently supported by more advanced capacity for rapid **presumptive and confirmatory-level testing** at state and large metropolitan public health laboratories. Further definitive characterization or highly specialized testing is provided by CDC, which serves as the national public health reference laboratory for major threat agents. The LRN consists of over 100 core and advanced capacity public health laboratories. In order to maintain network continuity, the respective State Public Health Laboratory Directors serve as the designated notification hub for maintaining operational integrity at the local level as well as communicating with CDC and FBI as appropriate.

Issues regarding the clinical use of threat agent assays: All of the biodetection assays and reagents utilized in the LRN, are intended for use in public health surveillance and the unique need related to the public health emergency, civilian biodefense and national security interests. These reagents are neither manufactured for commercial distribution nor provided for use in research purposes. An individual biodetection assay (and associated reagents) used in the standardized testing algorithm within the LRN should not be used to support a clinical diagnosis nor initiate a medical intervention without confirmation of the laboratory-based identification by another medically established diagnostic product or procedure.

Signs and Symptoms of Anthrax Infection

Inhalational anthrax: A brief prodrome resembling a viral respiratory illness followed by development of hypoxia and dyspnea, with radiographic evidence of mediastinal widening. This, the most lethal, form of anthrax results from inspiration of 8,000-40,000 spores of *B. anthracis*. The incubation of inhalational anthrax among humans is unclear, but it is reported to range between 1 and 7 days possibly ranging up to 60 days. Host factors, dose of exposure and chemoprophylaxis may play a role. Initial symptoms include sore throat, mild fever, muscle aches and malaise. These may progress to respiratory failure and shock. Meningitis frequently develops. Case-fatality estimates for inhalational anthrax are based on incomplete information regarding exposed populations and infected populations in the few case series and studies that have been published. However, case-fatality is extremely high, even with all possible supportive care including appropriate antibiotics. Records of industrially acquired inhalational anthrax in the United Kingdom before antibiotics were available reveal that 97% of cases were fatal. With antibiotic treatment the fatality rate is estimated to be at least 75%. Estimates of the impact of the delay in post-exposure prophylaxis or treatment on survival are not known. It has been suggested that each day of delay in initiating treatment may significantly increase morbidity and mortality from anthrax infection.

Gastrointestinal anthrax: Severe abdominal distress followed by fever and signs of septicemia. This form of anthrax usually follows the consumption of raw or undercooked contaminated meat and is considered to have an incubation period of 1-7 days. An oropharyngeal and an abdominal form of the disease have been described in this category. Involvement of the pharynx is usually characterized by lesions at the base of the tongue, sore throat, dysphagia, fever, and regional lymphadenopathy. Lower bowel inflammation usually causes nausea, loss of appetite, vomiting and fever, followed by abdominal pain, vomiting blood, and bloody diarrhea. The case-fatality rate is estimated to be 25-60%, and the effect of early antibiotic treatment on that case-fatality rate is not defined.

Cutaneous anthrax: A skin lesion evolving from a papule, through a vesicular stage, to a depressed black eschar. This is the most common naturally occurring type of infection (>95%) and usually occurs after skin contact with contaminated meat, wool, hides, or leather from infected animals. Incubation period ranges from 1-12 days. Skin infection begins as a small papule, progresses to a vesicle in 1-2 days followed by a necrotic ulcer. The lesion is usually painless, but patients also may have fever, malaise, headache and regional lymphadenopathy. The case fatality rate for cutaneous anthrax is 20% without, and less than 1% with, antibiotic treatment.

II. Anthrax Information for Laboratory Personnel

These guidelines provide background information and guidance to clinical laboratory personnel in recognizing *Bacillus anthracis* in a clinical specimen. They are NOT intended to provide training for laboratory identification of *B. anthracis*. Clinical lab personnel will most likely be the first ones to perform preliminary testing on clinical specimens from patients who may have been intentionally exposed to the organism, and will play a critical role in facilitating rapid identification of *B. anthracis*. Laboratory confirmation of *B. anthracis* should be performed at the State Public Health Laboratory.

Any suspected isolate of *B. anthracis* must be reported to the State Public Health Laboratory IMMEDIATELY. The State Public Health Laboratory is available for consultation or testing 24 hours per day and can be reached through the Department of Health Communicable Disease Epidemiology 24-hour emergency number. Following an appropriate consultation with the State Public Health Lab regarding a suspected isolate of *B. anthracis*, communication should then be established with the local FBI field office for possible law enforcement involvement.

HANDLING LABORATORY SPECIMENS (possible *B. anthracis*)

- Risk to lab personnel from handling clinical lab specimens with *B. anthracis* is low, but it is important to minimize possible exposures to personnel as well as prevent contamination of the lab. Standard lab practices are sufficient. If *B. anthracis* is suspected, these precautions should be followed:
 - Wear gloves and protective gowns when handling clinical specimens
 - Wash immediately with soap and water if there is direct contact with a clinical or lab specimen
 - Avoid splashing or creating aerosols
 - Perform lab tests in an annually certified Class 11 Biological Safety Cabinet; if that is not possible, then use standard lab protective eyewear and a mask
 - Blood cultures should be maintained in a closed system (blood culture bottles)
 - Keep culture plates covered at all times; minimize exposure when extracting specimens for testing
 - Work on a smooth surface that can be cleaned easily and wipe with bleach regularly.

- If lab or clinical specimen material is spilled or splashed onto lab personnel:
 - Remove outer clothing carefully while still in the lab and place in a labeled, plastic bag
 - Remove rest of clothing in the locker room and place in a labeled, plastic bag
 - Shower thoroughly with soap and water in the locker room
 - Inform your supervisor and physician

- If exposure to contaminated sharps occurs:
 - Follow standard reporting procedures for sharps exposures
 - Thoroughly irrigate site with soap and water and apply a disinfectant solution such as a 0.5% hypochlorite solution. DO NOT SCRUB AREA.
 - Promptly begin prophylaxis for cutaneous anthrax

- Recommended treatment for cutaneous exposure: prophylaxis with Ciprofloxacin 500 mg by mouth twice a day for 14 days or Doxycycline 100 mg by mouth twice a day for 14 days.
- Notify the State Department of Health (SDOH) and the State Public Health Laboratory (SPHL)

ROLE OF THE CLINICAL LABORATORY

- Perform laboratory tests for presumptive identification of *B. anthracis* on clinical specimens
- Raise your index of suspicion for *B. anthracis* when the clinical picture (provided by the clinician) involves a rapidly progressive respiratory illness of unknown cause in a previously healthy person
- Refer any suspected isolates IMMEDIATELY to the SDOH and SPHL

PRESUMPTIVE IDENTIFICATION OF *Bacillus anthracis*

- **Direct smears from clinical specimens**
 - Encapsulated broad rods in short chains, 2-4 cells. Gram stain can demonstrate clear zones (capsule) around rods. An India ink stain should be used to further visualize the capsule microscopically.
 - *B. anthracis* will not usually be present in clinical specimens until late in the course of the disease
- **Smears from sheep blood agar or other routine nutrient medium**
 - Non-encapsulated broad rods in long chains
 - When grown on nutrient agar in presence of 5% CO₂ or other basal media supplemented with 0.8% sodium bicarbonate, virulent strains will yield heavily encapsulated rods (Note: this procedure is performed in Level B laboratories).

Gram stain morphology of *B. anthracis*

- Broad, gram-positive rod: 1-1.5 x 3-5 μ
- Oval, central to subterminal spores: 1 x 1.5 μ with no significant swelling of cell
- Spores usually NOT present in clinical specimens unless exposed to atmospheric O₂

Colonial Characteristics of *B. anthracis*

- ***Bacillus anthracis* can be isolated primarily from blood, sputum, CSF, vesicular fluid or eschar, and stool (if gastrointestinal anthrax).**
- After incubation on a blood agar plate for 15-24 hours at 35-37 deg C, well isolated colonies are 2-5 mm in diameter; heavily inoculated areas may show growth in 6-8 hours
- Gray-white, flat or slightly convex colonies are irregularly round, with edges that slightly undulate, and have “ground glass” appearance
- Often have comma-shaped protrusions from colony edge (“Medusa head” colonies)
- Tenacious consistency (when teased with a loop, the growth will stand up like a beaten egg white)
- Non-hemolytic (weak hemolysis may be observed under areas of confluent growth in aging cultures and should NOT be confused with real β -hemolysis)

- Will not grow on MacConkey agar
- Non-motile

Presumptive Identification key for *Bacillus anthracis*

- Non-hemolytic
- Non-motile
- Encapsulated (requires India ink to visualize the capsule)
- Gram-positive, spore-forming rod

If *B. anthracis* is suspected

- The health care provider, local law enforcement, and the local and State DOH should be notified immediately
- Do not perform further tests once you have reason to suspect *B. anthracis*. The specimen should be transported to the DOH as directed (see Packaging and Transporting Protocol)
- Level B laboratories (State DOH) will perform the following presumptive and confirmatory tests:
 - lysis by gamma phage
 - capsule detection (by DFA)
 - detection of cell-wall polysaccharide antigen by DFA

DECONTAMINATION

- Effective sporicidal decontamination solutions
- **Commercially-available bleach, 0.5% hypochlorite (a 1: 10 dilution of household bleach)**
- Rinse off the concentrated bleach to avoid its caustic effects

Surfaces and non-sterilizable equipment

- Work surfaces should be wiped before and after use with a sporicidal decontamination solution
- Routinely clean non-sterilizable equipment with a decontamination solution

Contaminated instruments (pipettes, needles, loops, micro slides)

- Soak in a decontamination solution until autoclaving is performed

Accidental spills of material known or suspected to be contaminated with *B. anthracis*

For contamination involving fresh clinical samples:

- Flood with a decontamination solution
- Soak five minutes before cleaning up
- For contamination involving lab samples, such as culture plates or blood cultures, or spills occurring in areas that are below room temperature:
 - Gently cover spill, then liberally apply decontamination solution
 - Soak for one hour before cleaning up
 - Any materials soiled during the clean-up must be autoclaved or incinerated

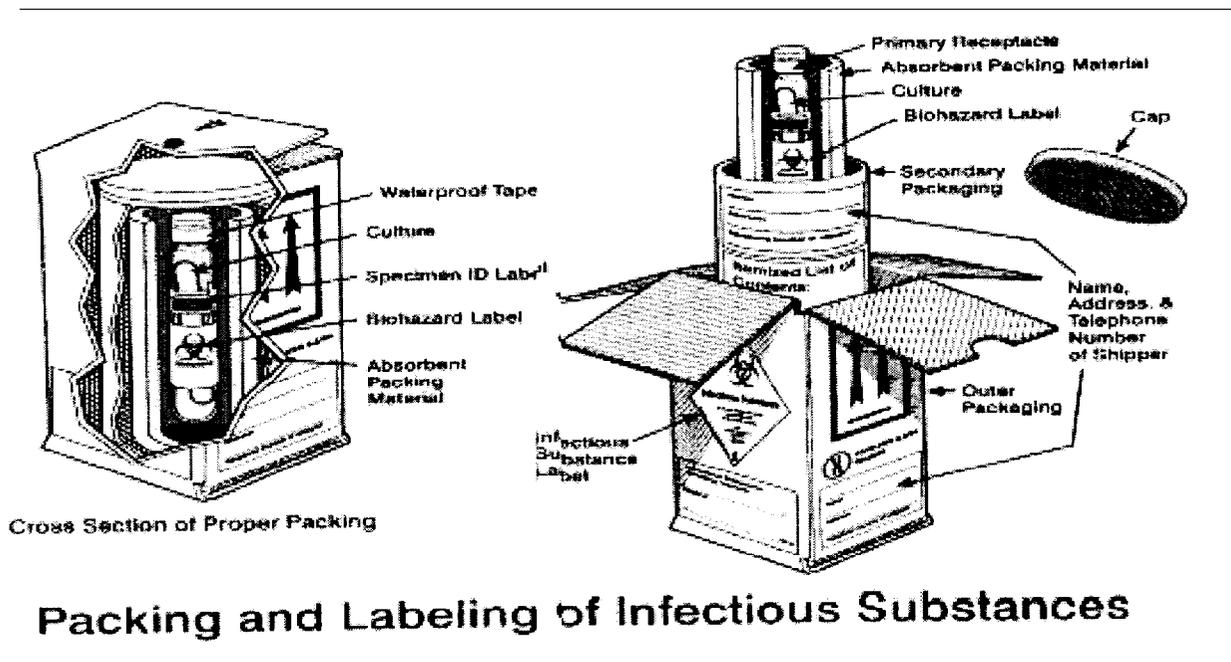
DISPOSAL

- Incinerate or steam-sterilize cultures, infected material, and suspect material

PACKAGING and TRANSPORTING PROTOCOL

Packaging and labeling specimens is the same as for any infectious substance

- If the specimen is a dry powder or paper material, place it in a plastic zip-lock bag, and place biohazard label (see diagram)
- If the specimen is a clinical specimen, place biohazard label on the specimen receptacle, wrap the receptacle with an absorbent material (see diagram)
- Place the bag or specimen receptacle into a leak proof container with a tight cover that is labeled “biohazard.”
- Place this container into a second leak proof container with a tight cover that is labeled “biohazard.” The size of the second container should be no larger than a one-gallon paint can.
- For a clinical specimen, an ice pack (not ice) should be placed in the second container to keep the specimen cold
- If the specimen is not a clinical specimen, but is paper or powder, the ice pack should be omitted
- Place the second container into a third leak proof container with a tight cover that is labeled “biohazard.” The third container should be no larger than a five-gallon paint can.
- Both containers should meet state and federal regulations for transport of hazardous material, and be properly labeled.



Transporting specimens to the DOH Public Health Lab

- Will be coordinated with the DOH Public Health Lab at [state telephone number]
- Local FBI personnel may be utilized to transport specimens if bioterrorism is suspected
- In cases where the specimen is shipped by commercial carrier, ship according to State and Federal shipping regulations

HELPFUL WEBSITES

- Biosafety in the Microbiology Lab www.cdc.gov/od/ohs
- Guideline for Isolation Precautions www.cdc.gov/ncidod/hip
- Public Health Image Library www.phil.cdc.gov
- World Health Organization (WHO): Guidelines for the Surveillance and Control of Anthrax in Humans and Animals
www.who.int/emc-documents/zoonoses/whoemczdi986c.html

REFERENCES for Laboratory Guidelines

- Laboratory protocols for clinical Laboratories for the identification of *Bacillus anthracis*. CDC BT public web site: www.bt.cdc.gov
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STANDARD OPERATING PROCEDURES
THE PRESUMPTIVE IDENTIFICATION OF
Bacillus an thracis

This protocol is designed to provide laboratories with techniques to identify microorganisms, in order to support clinicians in their diagnosis of potential diseases.

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I. Introduction

1. Overview

Anthrax is a zoonotic disease that is transmissible to humans through handling or consumption of contaminated animal products. The etiologic agent of anthrax, *Bacillus anthracis*, is a spore forming gram-positive bacillus. Although anthrax can be found globally in temperate zones, it is more often a risk in countries with less standardized and less effective public health programs. Areas currently listed as high risk are South and Central America, Southern and Eastern Europe, Asia, Africa, the Caribbean, and the Middle East. In these regions, herbivorous wildlife mammals, such as deer, wildebeest, elephants, and domesticated livestock, such as goats, sheep, cattle, horses, and swine, are at highest risk for disease. These animals usually become infected while grazing on contaminated land, eating contaminated feed or drinking from contaminated water holes. *B. anthracis* spores can remain viable in soil for many years. Anthrax infrequently occurs in livestock in North America; however, anthrax outbreaks have been reported among deer from Louisiana and Texas up through the Midwest and among wood buffalo in the Northwest Territory in Canada. Animal infections in the United States are reported most often in Texas, Louisiana, Mississippi, Oklahoma, and South Dakota. Birds, amphibians, reptiles, and fish are not directly susceptible to anthrax infection. However, some carnivorous mammals, such as dogs, lions, and omnivorous mammals such as swine, may be susceptible to anthrax infection through consumption of meat from infected animals.

2. Human infection

Humans can become infected with *B. anthracis* by handling products or consuming undercooked meat from infected animals. Infection may also result from inhalation of *B. anthracis* spores from contaminated animal products such as wool or the intentional release of spores during a bioterrorist attack. Human-to-human transmission has not been reported. Three forms of anthrax occur in humans: cutaneous, gastro-intestinal, and inhalational.

2.a. Cutaneous anthrax

Cutaneous infections occur when the bacterium or spore enters a cut or abrasion on the skin, such as when handling contaminated wool, hides, leather or hair products (especially goat hair) from infected animals. Skin infection begins as a raised itchy bump or papule that resembles an insect bite. Within 1-2 days, the bump develops into a fluid-filled vesicle, which ruptures to form a painless ulcer (called an eschar), usually 1-3 cm in diameter, with a characteristic black necrotic (dying) area in the center. Pronounced edema is often associated with the lesions because of the release of edema toxin by *B. anthracis*. 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 of respiratory distress caused by edema in the cervical and upper thoracic regions. Deaths are rare following appropriate antibiotic therapy, with lesions becoming sterile within 24 h and resolving within several weeks.

2.b. Gastrointestinal anthrax

The gastrointestinal form of anthrax may follow the consumption of contaminated meat from infected animals and is characterized by an 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 diarrhea. The mortality rate is difficult to determine for gastrointestinal anthrax but is estimated to be 25%-60%.

2.c. Inhalation anthrax

This form of anthrax results from inhaling *B. anthracis* spores, and is most likely following an intentional aerosol release of *B. anthracis*. After an incubation period of 1-6 days (depending on the number of inhaled spores), disease onset is gradual and nonspecific. Fever, malaise, and fatigue may be present initially, sometimes in association with a nonproductive cough and mild chest discomfort. These initial symptoms are often followed by a short period of improvement (ranging from several hours to days), followed by the abrupt development of severe respiratory distress with dyspnea (labored breathing), diaphoresis (perspiration), stridor (high-pitched whistling respiration), and cyanosis (bluish skin color). Shock and death usually occur within 24-36 h after the onset of respiratory distress, and in later stages, mortality approaches 100% despite

aggressive treatment. Physical findings are usually nonspecific. The chest X-ray is often pathognomonic (disease-specific) revealing a widened mediastinum with pleural effusions, but typically without infiltrates

B. anthracis can be detected by Gram stain of the blood and by blood culture with routine media, but often not until late in the course of the illness. Only vegetative encapsulated bacilli are present during infection. Spores are not found within the blood, partially because CO₂ levels in the body inhibit sporulation. Studies of inhalation anthrax in non-human primates (i.e., rhesus monkeys) showed that bacilli and toxins appear in the blood within 2-3 days of exposure. The appearance of toxins coincides with the appearance of bacilli in the blood, and tests are available to rapidly detect the toxins.

3. Antibiotic therapy

Most *B. anthracis* strains are sensitive to a broad range of antibiotics. Penicillin, ciprofloxacin, or doxycycline are usually recommended for the treatment of anthrax. To be effective, treatment should be initiated early. If left untreated, the disease is highly fatal.

4. Anthrax vaccine

An anthrax vaccine for humans is licensed for use in the United States. The vaccine is a cell-free filtrate that contains protective antigen and alum. The vaccine is reported to be 93% effective in protecting against cutaneous anthrax. Animal studies have suggested that the vaccine may also be protective against aerosol challenge. The anthrax vaccine is distributed by BioPort Corporation, Lansing, Mich.

B. anthracis is considered a potential biological warfare threat agent. The U.S. Department of Defense recommends anthrax vaccination of all U.S. active duty military personnel. According to the Advisory Committee for Immunization Practices (ACIP) civilians who should receive anthrax vaccine include persons who come in contact in the workplace with imported animal hides, furs, bonemeal, wool, animal hair (especially goat hair), and bristles and persons engaged in diagnostic or investigational activities which may put them in contact with anthrax spores. The vaccine should be administered only to healthy men and women aged 18-65 years since all studies to date have been conducted exclusively in that population. Pregnant women should not be vaccinated, because it is not known whether the anthrax vaccine can cause fetal harm.

The anthrax vaccination protocol consists of 3 subcutaneous injections given 2 weeks apart followed by 3 additional subcutaneous injections given at 6, 12, and 18 months. Annual booster injections of the vaccine are required to maintain immunity. Mild local reactions consisting of slight tenderness and redness at the injection site of the skin occur in approximately 30% of recipients. A moderate local reaction can occur if the vaccine is given to anyone with a past history of anthrax. Severe local reactions occur infrequently and consist of extensive swelling of the forearm in addition to the local reaction. Systemic reactions characterized by flu-like symptoms occur in fewer than 0.2% of vaccinees.

II. Laboratory Procedures for the identification of *Bacillus anthracis*

1. General

The procedures described below function to rule out presumptively identified *B. anthracis* in clinical specimens or isolates. These procedures should be performed in microbiology laboratories that utilize Biological Safety Level 2 (BSL 2) practices. Laboratory coats and gloves shall be worn when processing specimens and performing tests. Safety glasses or eye shields are recommended. Any activities that bring hands in contact with mucosal surfaces (for example, eating, drinking, smoking, or applying make-up) are prohibited. Hands should be washed before leaving the laboratory. Anthrax vaccination is not required.

Disclaimer. Names of vendors or manufacturers are provided as examples of suitable product sources and inclusion does not imply endorsement by the Centers for Disease Control and Prevention, the United States

Public Health Service, Department of Health and Human Services, the United States Army, or the Federal Bureau of Investigation.

1 .a. Handling of samples

For safety considerations, analysis of samples for biological threat agents is performed within a certified Class II biological safety cabinet (BSC). Procedures requiring removal of items from a BSC, such as slides for microscopy, should follow published microbiological practices and precautions. When using a BSC, assure that the cabinet does not contain unnecessary items that will interfere with proper airflow and function. As for any procedure involving infectious materials, standard personal protective gear should be used, such as latex gloves and laboratory coats, or disposable over garments. Additional respiratory protection should also be considered with materials or analytical procedures determined to be potentially hazardous outside the BSC. Once a biological agent has been identified, modifications in handling of samples can then be considered.

1. b. Decontamination

Commercially available household bleach solutions contain 5.25% hypochlorite and, when diluted 1: 10, are effective in routine decontamination of surfaces and instruments after working with *B. anthracis*. Contaminated items such as pipettes, needles, loops, and microscope slides should be immersed in decontamination solution until autoclaving. Work surfaces, such as a biological safety cabinet (BSC), should be wiped down before and after use with decontamination solution. The method of decontamination of a spill depends upon the nature of the spill. Spills involving fresh cultures or samples known to have low concentrations of spores should be flooded with decontamination solution and soaked for 5 min before cleanup. Spills that involve samples with high concentrations of spores, involve organic matter, or occur in areas of lower than room temperature (refrigerators, freezers) should be exposed to decontamination solution for at least 1 h before cleanup. Personnel involved in the cleanup of any spill should wear gloves, safety glasses, and a laboratory coat or gown during the cleanup process. Respiratory protection should be considered for spills in which a substantial aerosolization is suspected.

2. Collection of clinical specimens

2.a. Materials

Sporocidal disinfectant
Sputum cup
Sterile cotton swabs
Blood culture collection kit
Stool collection cup

2.b. Cutaneous anthrax

2.b.1. Vesicular stage: The organism is best demonstrated in this stage. Soak two dry sterile swabs in vesicular fluid from a previously unopened vesicle.

2.b.2. Eschar stage: Rotate two swabs beneath the edge of the eschar without removing the eschar.

2.c. Gastrointestinal anthrax

2.c.1. If the patient is able to produce a stool specimen, stool cultures should be performed. Obtain a stool culture.

2.c.2. In later stages of disease, blood cultures will yield the organism, especially if specimens are obtained prior to antibiotic treatment.

2.d. Inhalation anthrax

2.d. 1. If respiratory symptoms are present and sputum is being produced, obtain a specimen for culture and smear.

2.d.2. In later stages of disease (2-8 days post exposure) blood cultures may yield the organism, especially if specimens are drawn before antibiotic treatment.

3. Materials needed for processing of clinical specimens

5% Sheep blood agar plates [SBA]

MacConkey agar plates

Phenyl ethyl alcohol agar (PEA) plates (for stool specimens)

Trypticase soy broth

Clean glass microscope slides

Sterile cotton swabs (commercially available specimen transport swabs for aerobic culture are preferred)

Disposable bacteriologic inoculation loops

Clinical centrifuge with appropriate biocontainment tube holders

Sporicidal disinfectant (0.5% sodium hypochlorite or 0.5% calcium hypochlorite)

4. Isolation from clinical specimens

4.a. Sputum specimens

Inoculate 3 routine media for sputum specimens (i.e. SBA, MacConkey agar, broth enrichment)

4.b. Blood specimens

4.b. 1. Routine blood culture methods are sufficient.

4.b.2. There may be enough organisms in the blood to see them on direct smears by Gram stain. *B. anthracis* appears as short chains of 2-4 cells which are encapsulated as evidenced by clear zones around the bacilli. The presence of large encapsulated gram-positive rods in the blood is strongly presumptive for *B. anthracis* identification.

4.b.3. If blood culture bottle is positive, perform a Gram stain directly and observe for encapsulated rods. These blood cultures should also be subcultured to SBA and MacConkey agar plates.

4.c. Swab specimens

4.c. 1. Use one swab to inoculate 3 standard media for surface wounds (e.g., SBA, MacConkey agar, or broth enrichment).

4.c.2. Prepare a smear for Gram staining with the second swab.

4.d. Stool specimens

4.d. 1. Routine stool culture methods are sufficient (e.g., SBA, MacConkey agar, or PEA plates).

4.d.2. Do not use CVA or hectone agar plates.

4.e. CSF specimens

4.e. 1. If a clinical centrifuge with appropriate biocontainment tube holders is available, centrifuge the CSF specimen at 1500 X g for 15 minutes.

4.e.2. Collect the sediment and prepare a smear for Gram staining.

4.e.3. inoculate the remainder of the sediment onto SBA and broth enrichment media (tryptic soy broth or thioglycollate).

5. Incubation and examination of cultures

5.a. Cultures should be incubated at 35-37°C under ambient conditions.

5.b. Cultures should be examined within 18-24 h of incubation. Growth of *B. anthracis* may be observed as early as 8 h after inoculation.

6. Differential tests for the presumptive identification of *B. anthracis*

6.a. Colony characteristics of *B. anthracis*

6.a. 1. After incubation of SBA plates for 15-24 h at 35-37°C, well isolated colonies of *B. anthracis* are 2-5 mm in diameter. The flat or slightly convex colonies are irregularly round, with edges that are slightly undulate (irregular, wavy border), and have a ground-glass appearance. There are often comma-shaped projections from the colony edge, producing the "Medusa head" colony.

6.a.2. Colonies on SBA usually have a tenacious consistency. When teased with a loop, the growth will stand up like beaten egg white. In contrast to colonies of *B. cereus* and *B. thuringiensis*, colonies of *B. anthracis* are not β-hemolytic. However, weak hemolysis may be observed under areas of confluent growth in aging cultures and should not be confused with β-hemolysis.

6.a.3. When examining primary growth media, it is important to compare the extent of growth on SBA plates with that on MacConkey agar plates. *B. anthracis* grows well on SBA, but does not grow on MacConkey agar. *B. anthracis* grows rapidly; heavily inoculated areas may show growth within 6-8 h and individual colonies may be detected within 12-15 h. This trait can be used to isolate *B. anthracis* from mixed cultures containing slower-growing organisms.

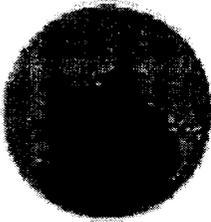


Figure 1. *B. anthracis* and *B. cereus* colony morphology. Overnight cultures of *B. cereus* (left side of plate) and *B. anthracis* (right side) on SBA.

6.b. Gram stain morphology of *B. anthracis*

6.b.1. Procedure

Perform Gram stain by usual procedures.

6.b.2. Interpretation of results

B. anthracis is a large gram-positive rod (1-1.5 X 3-5 μm) that forms oval, central-to-subterminal spores (1 X 1.5 μm) on SBA that do not cause significant swelling of the cell. Spores are not present in clinical samples unless exposed to atmospheric levels of CO₂; CO₂ levels within the body inhibit sporulation. Vegetative cells seen on Gram stain of blood and impression smears are in short chains of 2-4 cells that are encapsulated. However, cells from growth on SBA under ambient conditions, are not encapsulated and occur as long chains of bacilli. When grown on nutrient agar in the presence of 5% CO₂ or on other basal media supplemented with 0.8% sodium bicarbonate, virulent strains will yield heavily encapsulated bacilli. The capsule can be visualized microscopically using India Ink.



Figure 2. Gram stain of *B. anthracis* from SBA. Magnification 1,000X.

6.c. India ink staining of clinical samples (blood and CSF) for capsule

6.c.1. Purpose

India ink is useful for improving visualization of encapsulated *B. anthracis* in clinical samples such as blood, blood culture bottles, or cerebrospinal fluid (CSF).

6.c.2. Materials

Microscope slides

Cover glasses

India ink (Bactidrop, Remel, Inc., Catalog # 21-518 or equivalent)

Microscope with 100X oil immersion objective

6.c.3. Controls

a) Control strains

(1) Positive control: *Klebsiella pneumoniae* on SBA or equivalent

(2) Negative control: *E. coli* ATCC 25922 or equivalent

b) Method controls: Perform the test with suspensions of fresh cultures of the control strains. Control strains should be assayed on each day of testing.

c) Resolving an out-of-control result: check the purity and identity of the control strains and repeat the test.

6.c.4. Procedure

a) For the controls, transfer a small amount of growth (1 mm diameter) from each control SBA plate into 0.5 ml whole EDTA-treated blood or serum. Mix.

b) For the unknowns, take 100 ml of sample (blood, CSF)

c) Transfer 5-10 ml of unknown or control to a slide, place a cover glass on the drop, and then add 5-10 ml of India ink to the edge of the cover glass.

d) After the ink diffuses across, view the cells using 100X oil immersion objective with oil on top of the cover glass.

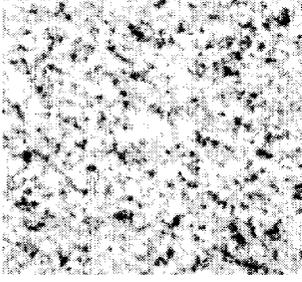


Figure 3. India ink staining of *B. anthracis*

6.c.5. Interpretation of results

The capsule will appear as a well-defined clear zone around the cells for the positive control. No zone should be present in the negative control.

ACKNOWLEDGEMENTS:

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6.d. Motility test: Wet mount or motility medium

6.d.1. Purpose

This test determines the motility of suspect isolates. *B. anthracis* is a nonmotile species. This characteristic is unusual among *Bacillus* species and is therefore useful in the preliminary identification of *B. anthracis* isolates. Two methods are given: the wet mount and the tubed motility test.

6.d.2. Materials

a) For wet mount procedure

Precleaned microscope slides
Cover glasses
Sterile distilled water
Disposable bacteriologic inoculating loop
Light microscope with 40X objective and 1 OX eyepiece
Sterile glass tube

b) For tubed motility test

Tubed motility media (Remel, Inc., Catalog # 06-1408 or equivalent), 5 ml per tube
Sterile disposable 1 ml inoculating loop or needle

6.d.3. Controls

a) Control strains

(1) Positive control: *Pseudomonas aeruginosa* ATCC 35032 or equivalent
(2) Negative control: *Acinetobacter* spp. ATCC 49139 or equivalent

b) Method controls: Perform the test with fresh cultures of the control strains using the same method as with unknowns. Control strains should be assayed on each day of testing.

c) Resolving an out-of-control result: Check the purity and identity of the control strains and repeat the test.

6.d.4. Procedures

Wet mount and motility

a) Wet mount procedure

- (1) Deliver 2 drops (approximately 0.1 ml) of sterile distilled water into the sterile glass tube.
- (2) Using the inoculating loop, sample a suspicious colony from a 12-20 h culture and suspend the growth in the water. (Alternatively, a loopful of medium from a fresh broth culture can be used).
- (3) Transfer 1 drop of the suspension to the microscope slide and overlay with the cover glass.
- (4) Examine the slide under the microscope using the 40X objective (total magnification = 400X).
- (5) Discard slides in 0.5% hypochlorite solution.

b) Motility test medium procedure

- (1) Using the sterile inoculating needle, remove some growth from an isolated, suspicious colony of an 18-24 h culture.
- (2) Inoculate the motility tube by carefully stabbing the needle 3-4 cm into the medium and then drawing the needle directly back out so that a single line of inoculum can be observed.
- (3) Incubate the tube aerobically at 35-37°C for 18-24 h.

c) Interpretation of results

- (1) For wet mount: Motile organisms can be observed moving randomly throughout the suspension. Nonmotile organisms either fail to move or move with Brownian motion.
- (2) For motility test medium: Nonmotile organisms, such as *B. anthracis*, will form a single line of growth that does not deviate from the original inoculum stab. Motile organisms will form a diffuse growth zone around the inoculum stab.

7. Presumptive identification key for *B. anthracis*

7.a. From clinical samples, such as blood, CSF, or lesion material: encapsulated gram-positive rods.

7.b. Gram-positive, broad rod, spore-positive: *Bacillus* species.

7.c. Spores are nonswelling and oval shaped; ground glass appearance of colonies: *Bacillus* morphology group 1 (includes *B. anthracis*, *B. cereus*, *B. thuringiensis*, and *B. cereus* var. *mycoides*)

7.d. Nonmotile: *B. anthracis* and *B. cereus* var. *mycoides*

7.e. Nonhemolytic: presumptive *B. anthracis*

8. Actions if a presumptive *B. anthracis* colony is identified and suspected as a bioterrorist threat agent

8.a. Preserve original specimens pursuant to a potential criminal investigation.

8.b. Contact local FBI, state public health laboratory, and state public health department.

8.c. Local FBI agents will forward isolates to a State health department laboratory as is necessary.

Consultation with a state health department laboratory is strongly encouraged as soon as *B. anthracis* is suspected as a bioterrorist threat agent.

9. Listed vendors

American Type Cell Culture [ATCC], 800-638-6597 Remel, Inc., 800-255-6730

Becton-Dickinson Bioscience [BD], 800-675-0908

III. Appendix

Bacillus anthracis ID Overview Flowchart

Suspected Identification of
Bacillus anthracis:
Clinical findings (mediastinitis, septicemia, possibly
lesions)

Basis for Presumptive Identification of *B.*
anthracis: and notification of authorities:
Nonhemolytic, nonmotile, aerobic, spore-
forming Gram-positive rod. Most likely
isolated from blood.

**REFER CULTURE TO LEVEL B OR
LEVEL C LABORATORY**

Notify Chief, Pathology; Chief, Preventive Medicine; Infectious Disease Service that ID is pending on a presumptive *B. anthracis*.; (Your Preventive Medicine staff will notify your Commander, Local FBI, Local Public Health Laboratory, and State Public Health Department, as needed)