SENTINEL LEVEL CLINICAL LABORATORY GUIDELINES

FOR

SUSPECTED AGENTS OF BIOTERRORISM

AND

EMERGING INFECTIOUS DISEASES

Botulinum Toxin

American Society for Microbiology (ASM)

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PREANALYTICAL CONSIDERATIONS

I. PRINICIPLE

A. Introduction

Clostridium botulinum organisms are straight to slightly curved, gram-positive (in young cultures), motile, anaerobic rods, 0.5 to 2.0 µm in width and 1.6 to 22.0 µm in length, with oval, subterminal spores. *Clostridium botulinum* is a Select Agent Tier 1 organism. Sentinel Clinical Laboratories will not isolate nor identify *C. botulinum* or its toxins. In suspect cases of botulism, Sentinel Clinical Laboratories will obtain appropriate specimens, ensure that they are packaged safely, and consult with the patient's physician, their designated LRN Reference Laboratory and the CDC. Sentinel Clinical laboratories are defined as those certified to perform high complexity testing under the Clinical Laboratory Improvement Amendments of 1988 (CLA) by the Centers for Medicare and Medicaid services 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 do not require registration with the Select Agent Program to conduct diagnostic testing for Select Agents, both Tier I and non-Tier I. Testing for Select Agents may be performed by Sentinel Clinical Laboratories as long as the laboratory destroys any residual specimen and destroys or transfers the confirmed select agent with 7 days of identification. Reporting of all identified Select Agents is still required; laboratories will need to complete Form 4. If the organism is transferred following identification, then the laboratory must also 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 at www.selectagents.gov.

Clostridium botulinum are a group of anaerobic organisms, commonly found in soils and aquatic habitats throughout the world, that are alike only in that they are clostridia and produce antigenically distinct neurotoxins with similar pharmacologic actions. The seven types of *C. botulinum* (A to G) are distinguished by the antigenic characteristics of the neurotoxins they produce. Human botulism is primarily caused by the strains of *C. botulinum* that produce toxin types A, B, and E, but rare cases of type F have been reported. Also, rare cases of human botulism by *C. butyricum*-like and *C. baratii*-like organisms have been reported to produce botulinum type E and F toxins, respectively. *C. botulinum* type G, which has been reclassified as *Clostridium argentinense*, has at the case report level been suggested as a cause of sudden, unexpected death, but a clearly causal relationship has not been established.

Worldwide, sporadic cases and small outbreaks occur where food products are prepared or preserved by methods that do not destroy the spores and permit toxin formation. Cases rarely result from commercially processed products, but outbreaks have occurred through cans that were damaged after processing. The potential for intentional poisoning with botulinum toxin is now a realistic threat. Inhalation and foodborne botulism are the likely forms of disease following a bioterrorist event. It is estimated that as little as 1 g of aerosolized botulinum toxin has the potential to kill at least 1.5 million people. Botulinum toxin is absorbed through the lungs and into the bloodstream. Three cases of human inhalational botulism were reported in

1962 in veterinary technicians in Germany who were working with aerosolized botulinum toxin in animals. Symptoms occurred approximately 72 h after exposure.

Past efforts to weaponize botulinum toxin include a U.S. weapons program beginning in World War II and ending after the 1972 Biological and Toxin Weapons Convention, research conducted in the former Soviet Union and Iraq as late as the 1990s, and the attempted use of aerosolized botulinum toxin in Japanese cities by the Aum Shinrikyo cult on at least three occasions in the 1990s.

It is anticipated that in a bioterrorism related exposure, botulinum toxin would most likely be transmitted by ingestion of toxin-contaminated food. Contamination of a municipal water supply is unlikely, since it would require a large quantity of toxin. Moreover, toxin is naturally inactivated in freshwater within 3 to 6 days and rapidly (within 20 min) inactivated by standard municipal potable water treatments.

In April 2002, the FDA approved the use of botulinum toxin type A for cosmetic purposes. Therapeutic botulinum toxin contains about 0.3% of the estimated lethal human inhalation dose and only 0.005% of the estimated lethal human oral dose. Therefore, this form of toxin is not likely to be used as a bioweapon.

B. Geographic Distribution

C. botulinum spores are ubiquitous in soil worldwide. Approximately 100 cases are reported in the U.S. each year. Five western states (California, Washington, Colorado, Oregon, and Alaska) have accounted for more than half of all reported foodborne outbreaks since 1950. Alaska alone accounts for 16% of these outbreaks, due in great part to the consumption of fermented seafood, seals, whales, and other mammal meat products contaminated with toxin-producing clostridia.

C. Disease and Clinical presentation

Botulism is a muscle-paralyzing disease caused by a toxin made by a bacterium called *Clostridium botulinum*. Four distinct forms of botulism have occurred in humans: (i) foodborne, (ii) wound, (iii) infant, and (iv) child, or adult non-foodborne. Foodborne botulism results from the ingestion of food containing preformed toxin. Wound botulism is caused by organisms that multiply and produce toxin in a contaminated wound. This occurs most commonly in injection drug users. Infant botulism is due to the endogenous production of toxin by germinating spores of *C. botulinum* in the intestine of the infant. Child or adult botulism is represented by those cases in which no food vehicle can be identified, there is no evidence of wound botulism, and there is the possibility of intestinal colonization in a person older than 1 year of age. Important epidemiologic features and some clinical characteristics distinguish the types of botulism that cause human illness.

The clinical syndrome of botulism is dominated by the neurologic symptoms and signs resulting from a toxin-induced blockade of the voluntary motor and autonomic cholinergic junctions. Incubation periods for foodborne botulism are reported to be as short as 6 h or as long as 10 days, but generally the time between toxin ingestion and onset of symptoms ranges from 18 to 36 h. The ingestion of other bacteria or their toxins in improperly preserved food or changes in

bowel motility are likely to account for the abdominal pain, nausea, vomiting, and diarrhea that often precede or accompany the neurologic symptoms of foodborne botulism. Dryness of the mouth, inability to focus to a near point (prompting the patient to complain of "blurred vision"), and diplopia (double vision) are usually the earliest neurologic complaints. If the disease is mild, no other symptoms may develop, and the initial symptoms will gradually resolve. The person with mild botulism may not come to medical attention. In more severe cases, however, these initial symptoms may be followed by voice impairment (dysphonia, dysarthria), difficulty swallowing (dysphagia), and peripheral muscle weakness. If illness is severe, respiratory muscles become involved, leading to respiratory failure and death unless supportive care is provided. Recovery follows the regeneration of new neuromuscular connections. A 2- to 8-week duration of respiratory support is common, although patients have required respiratory support for up to 7 months before the return of muscular function. Death occurs in 5 to 10% of cases of foodborne botulism; early deaths result from a failure to recognize the severity of disease or from secondary pulmonary or systemic infections, whereas deaths after 2 weeks are usually from the complications of long-term mechanical respiratory management.

Animal studies have shown that botulinum toxins produce similar effects whether inhaled or ingested. Presumably the gastrointestinal symptoms present in foodborne botulism would be absent following inhalation. The onset of symptoms of inhalational botulism in animals extends from 24 h to 2 days, depending on the extent of exposure.

The administration of antitoxin is the only specific therapy available for botulism, and evidence suggests that it is effective only if given very early in the course of neurologic dysfunction. Thus, the diagnosis of this illness cannot await the results of studies that may be long delayed and only confirmatory in some cases. The diagnosis and the decision to treat should be made on the basis of the case history and physical findings.

Botulism is not transmitted from person to person. However, even a single diagnosis should be considered a possible public health emergency situation due to the possibility of other common source cases. Since there are cases that are acquired in the absence of bioterrorism, clinicians should look for clusters of cases of an acute onset, afebrile, symmetric, descending flaccid paralysis that begins in the bulbar muscles and includes dilated pupils and dry mucous membranes but normal mental status and an absence of sensory changes.

D. Case Definition

The Centers for Disease Control and Prevention have established case definitions based on each form of botulism (CDC 2011 case definition: http://wwwn.cdc.gov/NNDSS/script/casedef.aspx?CondYrID=622&DatePub=1/1/2011). When considering foodborne botulism, a probable case is one that is a clinically compatible case with an epidemiologic link (e.g., ingestion of a home-canned food within the previous 48 hours). A confirmed case is one that is a clinically compatible case that is laboratory confirmed or that occurs among persons who ate the same food as persons who have laboratory-confirmed botulism. Laboratory confirmation is the detection of botulinum toxin in serum, stool, or patient's food, or the isolation of *Clostridium botulinum* from stool. This case definition is also used for adult and child non-foodborne cases.

The case definition for infant botulism is only for a confirmed case. A case is confirmed if it is a clinically compatible case that is laboratory-confirmed, occurring in a child aged less than 1 year. Laboratory confirmation is the detection of botulinum toxin in stool or serum, or the isolation of *Clostridium botulinum* from stool

Wound botulism has case definitions for both probable and confirmed cases. A probable case is one that is a clinically compatible case in a patient who has no suspected exposure to contaminated food and who has either a history of a fresh, contaminated wound during the 2 weeks before onset of symptoms, or a history of injection drug use within the 2 weeks before onset of symptoms. A confirmed case is one that is a clinically compatible case that is laboratory confirmed in a patient who has no suspected exposure to contaminated food and who has a history of a fresh, contaminated wound during the 2 weeks before onset of symptoms, or a history of injection drug use within the 2 weeks before onset of symptoms. Laboratory confirmation is detection of botulinum toxin in serum, or isolation of *Clostridium botulinum* from the wound.

E. Presumptive diagnosis

A presumptive diagnosis is made based on the clinical picture in the absence of other etiologies. Botulism needs to be differentiated from other neurological diseases, including Landry-Guillain-Barre syndrome, tick paralysis, myasthenia gravis, and Lambert-Eaton syndrome (an autoimmune disease that causes muscle weakness and easy fatigability, particularly in the pelvic muscles and thighs).

Once a presumptive clinical diagnosis is made, an intense epidemiologic investigation should ensue to identify other related cases. The diagnosis will be confirmed based clinical features and laboratory results.

II SAFETY CONSIDERATIONS

A. Handling of samples for identification

Botulism toxins are extremely poisonous. Exposure to the toxin represents the primary laboratory hazard. The 50% lethal dose of this toxin is 0.001 g/kg of body weight; therefore acquisition by ingestion, inhalation or absorption can cause intoxication and death. All materials suspected of containing toxin must be handled using standard precautions and BSL 2 containment criteria.

B. Precautions

Additional primary containment and personnel precautions, such as those recommended for BSL 3, are indicated for activities with a high potential for aerosol or droplet production (5,6) A class II biologic safety cabinet should be used when processing specimens to prevent the release of aerosols in the laboratory**.**

C. Decontamination procedures

C. botulinum is inactivated by a freshly prepared 1:10 dilution of household bleach. The disinfectant must be in contact with the toxin or organism for 15 to 20 minutes to ensure complete inactivation. If a spill is suspected of containing both the toxin and the organism, it should be treated sequentially with bleach and 1 M sodium hydroxide.

Note: Exposure to bleach does not inactivate spores of *C. botulinum*.

III. Materials

A. Supplies

i. Anaerobic transport vials

ii. Leak-proof containers (i.e., sealed plastic bags and other plastic containers) Packaging materials. Refer the ASM Packing and Shipping Sentinel Guidelines at http://clinmicro.asm.org/index.php/bench-work-resources/conducting-daily-operations/packaging-and-shipping

IV Specimen Collection and Transport

A. Specimen Considerations

The suspicion of botulism is a public health emergency: notify both local public health officials and the state public health laboratory for approval to submit samples for testing. DO NOT attempt to culture, identify the organism, or perform toxin analysis.

NOTE: Sentinel laboratories may conduct routine wound cultures prior to submitting specimen to an LRN Reference laboratory for botulism testing.

Collection and Transport of Clinical Specimens for Laboratory Testing and Confirmation	
Collect all specimens prior to treatment with antitoxin. **Note:** Treatment should not wait for laboratory confirmation.	
Serum	• Use red top or serum separator tubes to obtain serum (no anticoagulant). • Collect enough blood to provide at least 10 ml of serum for mouse toxicity tests (usually 20 ml of whole blood). • Store specimens at 4°C. • Ship on cold packs as soon as possible. • Serum volumes less than 3 ml will provide inconclusive results. • Whole blood should not be sent as it typically undergoes excessive hemolysis during transit.
Feces	• Collect 10 to 50 grams of feces. • Place into sterile unbreakable container and label carefully. • No transport media. • Store specimens at 4°C. • Ship on cold packs as soon as possible.
Enema	• Collect 20 ml. • Place in a sterile unbreakable container and label carefully. • Store specimens at 4°C. • Ship on cold packs as soon as possible.

	• If an enema is given because of constipation, a minimal amount of fluid (preferably sterile, nonbacteriostatic water) should be used to obtain the specimen so that the toxin will not be unnecessarily diluted.
Gastric or Vomitus	• Collect 20 ml • Place in sterile unbreakable container and label carefully. • Store specimens at 4°C. • Ship on cold packs as soon as possible.
Food Samples	• Foods should be left in their original containers if possible. • If transferring, place food into a sterile unbreakable, puncture resistant container and labeled carefully. • Place containers individually in leak-proof containers (e.g., sealed plastic bags) to prevent cross-contamination during shipment. • Store specimens at 4°C. • Ship on cold packs as soon as possible. • Empty containers with remnants of suspected foods can be examined.
Tissue, Exudate or Wound Swab	• Place specimen into sterile unbreakable container and label carefully. • Specimens should be placed in anaerobic transport media. • Store and transport without refrigeration.
Autopsy Specimens	• Feces, gastric contents and serum if available. • Collect feces from different levels of both large and small intestine. • Transport all specimens as described above.

B Submitting Specimens

Due to the inherent dangers in working with *C. botulinum*, Sentinel laboratories are responsible only for the collection, packaging and shipment of specimens suspected of containing botulinum toxin. Submit all samples to LRN Reference laboratory approved to perform testing as directed by the state public health laboratory. Note that not all states currently have testing capability. Toxin testing is performed primarily by a mouse toxicity and toxin neutralization method. Final results may be expected in 8 to 14 days.

C. Limitations

If the patient has been on any medication that might interfere with toxin assays or culturing of the stool, the laboratory should be notified. For example, it has been demonstrated that anticholinesterase drugs given orally to patients for myasthenia gravis can interfere with mouse botulinum toxin assays of stool extracts.

D. Rejection criteria

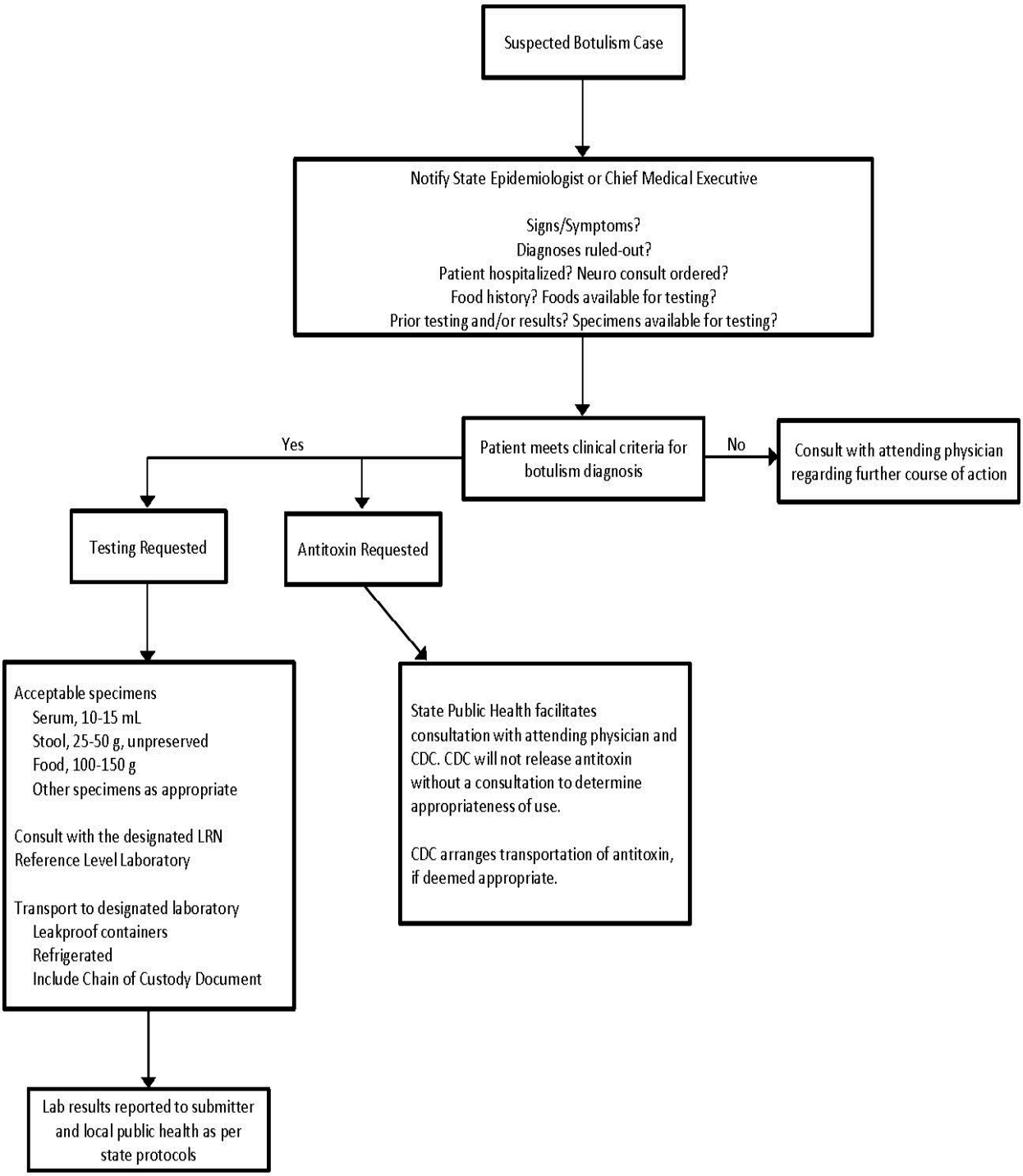
i. Use established laboratory criteria.
ii. Environmental or non-clinical samples are not processed by Sentinel laboratories; contact your designated LRN Reference Laboratory or state public health laboratory directly.

E. Shipping.

Refer to ASM procedure in the ASM Packing and Shipping Sentinel Guidelines at http://clinmicro.asm.org/index.php/bench-work-resources/conducting-daily-operations/packaging-and-shipping

ANALYTICAL CONSIDERATIONS

Not all LRN Reference Laboratories have the ability to test for *Clostridium botulinum* or botulinum toxin. Contact your designated LRN Reference Laboratory for specific instructions.


Suspected Botulism Case
Notify State Epidemiologist or Chief Medical Executive
Signs/Symptoms?
Diagnoses ruled-out?
Patient hospitalized? Neuro consult ordered?
Food history? Foods available for testing?
Prior testing and/or results? Specimens available for testing?
Patient meets clinical criteria for botulism diagnosis
Yes
No
Consult with attending physician regarding further course of action
Testing Requested
Antitoxin Requested
Acceptable specimens
Serum, 10-15 mL
Stool, 25-50 g, unpreserved
Food, 100-150 g
Other specimens as appropriate
Consult with the designated LRN Reference Level Laboratory
Transport to designated laboratory
Leakproof containers
Refrigerated
Include Chain of Custody Document
State Public Health facilitates consultation with attending physician and CDC. CDC will not release antitoxin without a consultation to determine appropriateness of use.
CDC arranges transportation of antitoxin, if deemed appropriate.
Lab results reported to submitter and local public health as per state protocols

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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

V. Reporting and Notification

i. Immediately notify your designated LRN Reference Laboratory; they will provide the referring laboratory with guidance and recommendations for retaining the specimen or isolate.
ii. Once the identification is confirmed, the Sentinel Laboratory is required to destroy the specimen or isolate (e.g., autoclaving). In particular, the appropriate material, including blood culture bottles, tubes and plates and actual clinical specimens (serum, feces, vomitus, food, etc.) should be saved in a secure location until the Reference Laboratory confirms the identification.
iii. Notify other public health authorities as required by local and state communicable disease reporting requirements if *C. botulinum* is suspected. The state public health laboratory/state public health department will notify law enforcement officials (state and federal). Full identification and susceptibility testing must not be performed by the Sentinel Laboratory.
iv. Do not ship specimens to the LRN Reference Laboratory without prior arrangements.
v. Within the hospital setting, the infection preventionists and/or infectious disease service should be notified so further investigation of the patient's history can be made.

Sentinel Laboratories should consult with the designated LRN Reference Laboratory prior to or concurrent with testing, if culture for C. *botulinum* 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). FBI and state public health laboratory/state public health department with coordinate the transfer of isolates/specimens to a higher-level LRN laboratory as appropriate.

If *C. botulinum* is ruled out, proceed with efforts to identify using established procedures.

If other cases are suspected or there is a laboratory exposure, collect samples to submit to the designated LRN Reference Laboratory for testing.

VI. Summary and Special Considerations

A. Treatment

On March 13, 2010, a new heptavalent botulinum antitoxin (HBAT, Cangene Corporation) was released through a CDC-sponsored Food and Drug Administration (FDA) Investigational New Drug (IND) protocol replacing a licensed bivalent botulinum antitoxin AB and an investigational monovalent botulinum antitoxin E (BAT-AB and BAT-E, Sanofi Pasteur). HBAT is the only botulinum antitoxin available in the United States for noninfant botulism.

HBAT contains equine-derived antibody to all seven known botulinum toxin types (A--G) with the following nominal potency values: 7,500 U anti-A; 5,500 U anti-B; 5,000 U anti-C; 1,000 U anti-D; 8,500 U anti-E; 5,000 U anti-F; and 1,000 U anti-G. Repeat HBAT dosing might be indicated for some wound or intestinal colonization patients. Prior to administration of antitoxin, patients should be skin tested for sensitivity to equine serum. The HBAT FDA IND treatment protocol includes specific, detailed instructions for intravenous administration of antitoxin and return of required paperwork to CDC.

BabyBIG (botulism immune globulin) remains available for infant botulism through the California Infant Botulism Treatment and Prevention Program. BabyBIG consists of human-derived botulism antitoxin antibodies and is approved by FDA for the treatment of infant botulism types A and B.

Supportive therapy, including the use of a ventilator, may be required if the patient's clinical course includes respiratory failure and paralysis.

Additional information regarding CDC's botulism treatment program is available at http://www.bt.cdc.gov/agent/botulism.

B. Select Agent Reporting and Compliance

i. Reporting of all identified Select Agents is still required, even though Sentinel laboratories are not required to register under the Select Agent Rule.
ii. 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
iii. 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.
iv. 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

i. Cultures and clinical materials should be autoclaved.
ii. If no autoclave is available, contaminated items should be soaked in 10% bleach or 10% formalin for 24 hours.

D. Packaging and Shipping

i. Refer to the ASM Packing and Shipping Sentential Guidelines.

ii. All materials sent to your designated LRN Reference Laboratory must be shipped in compliance with IATA and DOT regulations.

REFERENCES

1. **Stevens, D.L., A.E. Bryant, A. Berger and C. von Eichel/Streiber.** 2011. *Clostridium*. p. 834-857. *In* J. Versalovic, K.C. Carroll, G. Funke, J.H.Jorgensen, M.L. Landry and D.W. Warnock (ed.), Manual of Clinical Microbiology, 10th ed. ASM Press, Washington, D.C.
2. **Arnon, S. S.** 1992. Infant botulism, p. 1095-1102. *In* R. Feigen and J. Cherry (ed.), Textbook of pediatric infectious diseases. W. B. Saunders, Philadelphia, PA.
3. **Cato, E. P., W. L. George, and S. M. Finegold.** 1986. Genus *Clostridium*, Praemozski 1880, 23AL, p. 1141-1200. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharp, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2.Williams & Wilkins, Baltimore, MD.
4. **Centers for Disease Control and Prevention.** 1976. Follow-up: botulism associated with commercial cherry peppers. Morb. Mortal. Wkly. Rep. **25:**148.
5. **Centers for Disease Control and Prevention.** 1998. Botulism in the United States, 1899-1996. Handbook for epidemiologists, clinicians, and laboratory workers. Centers for Disease Control and Prevention, Atlanta, GA.
6. **Danzig, R.,** 1996. Biological warfare: a nation at risk–a time to act. Strategic Forum **58:**1-4.
7. **Eitzen, E., J. Pavlin, T. Cieslak, G. Christopher, and R. Culpepper.** July 1998. Medical management of biological casualties handbook, 3rd ed. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.
8. **Ekeus, R.** 1991. Report of the Secretary General on the status of the implementation of the Special Commission's plan for the ongoing monitoring and verification of Iraq's compliance with relevant parts of Sector C of Security Council Resolution 687, United Nation's Special Commission (UNSCOM). United Nations, New York, N.Y.
9. **Gilchrist, M.J.R., W.P. McKinney, J.M. Miller and A.S. Weissfeld**. 2000. *Cumitech 33, Laboratory Safety, Management and Diagnosis of Biological Agents Associated with Bioterrorism*. Coordinating ed., J.W. Snyder. ASM Press, Washington, DC.
10. **Hatheway, C. L.** 1995. Botulism: the present status of the disease. Curr. Top. Microbiol. Immunol. **195:**55-75.
11. **Hughes, J. M., J. R. Blumenthal, M. H. Merson, G. L. Lombard, V. R. Dowell, Jr., and E. J. Gangarosa.** 1981. Clinical features of types A and B foodborne botulism. Ann. Intern. Med. **95:**442-445.
12. **Long, S. S., J. L. Gajewski, L. W. Brown, and P. H. Gilligan.** 1985. Clinical, laboratory, and environmental features of infant botulism in southeastern Pennsylvania. Pediatrics **75:**935-941.
13. **Merson, M. H., and V. R. Dowell, Jr.** 1973. Epidemiologic, clinical and laboratory aspects of wound botulism. N. Engl. J. Med. **289:**1105-1110.
14. **Miller, J.M**. 2000. The laboratory response to agents of bioterrorism. American Society for Microbiology Audioconference Series. American Society for Microbiology, Washington, DC.
15. **Shapiro, R. L., C. Hatheway, S. Becher, and D. L. Swerdlow.** 1997. Botulism surveillance and emergency response. A public health strategy for a global challenge. JAMA **278:**433-435.

16. **Shapiro, R. L., C. Hatheway, and D. L. Swerdlow.** 1998. Botulism in the United States: a clinical and epidemiologic review. Ann. Intern. Med. **129:**221-228.
17. **St. Louis, M. E., S. H. Peck, D. Bowering, G. B. Morgan, J. Blatherwick, S. Banerjee, G. D. Kettyls, W. A. Black, M. E. Milling, A. H. Hauschild et al.** 1988. Botulism from chopped garlic: delayed recognition of a major outbreak. Ann. Intern. Med. **108:**363-368.
18. **Tackett, C. O., W. X. Shandera, J. M. Mann, N. T. Hargrett, and P. A. Blake.** 1984. Equine antitoxin use and other factors that predict outcome in type A foodborne botulism. Am. J. Med. **76:**794-798.
19. **Terranova, W., J. G. Breman, R. P. Locey, and S. Speck.** 1978. Botulism type B: epidemiologic aspects of an extensive outbreak. Am. J. Epidemiol. **108:**150-156.
20. **Woodruff, B. A., P. M. Griffin, L. M. McCroskey, J. F. Smart, R. B. Wainwright, R. G. Bryant, L. C. Hutwagner, and C. L. Hatheway.** 1992. Clinical and laboratory comparison of botulism from toxin types A, B, and E in the United States 1975-1988. J. Infect. Dis. **166:**1281-1286.

Suggested Reading

- **Infectious Diseases Society of America.** 2002. Bioterrorism information and resources. http://www.idsociety.org.