

**SENTINEL LABORATORY GUIDELINES
FOR
SUSPECTED AGENTS OF BIOTERRORISM**

Botulinum Toxin

American Society for Microbiology

Credits: Botulinum Toxin

Subject Matter Experts, ASM

Daniel S. Shapiro, M.D.
Boston Medical Center
Boston, MA

Alice S. Weissfeld, Ph.D.
Microbiology Specialists Incorporated
Houston, TX

ASM Laboratory Protocol Working Group

Vickie Baselski, Ph.D.
University of Tennessee at Memphis
Memphis, TN

Roberta B. Carey, Ph.D.
Loyola University Medical Center
Maywood, IL

Peter H. Gilligan, Ph.D.
University of North Carolina
Chapel Hill, NC

Larry Gray, Ph.D.
TriHealth Laboratories and
University of Cincinnati College of Medicine
Cincinnati, OH

Rosemary Humes, MS, MT (ASCP) SM
Association of Public Health Laboratories
Washington, DC

Karen Krisher, Ph.D.
Clinical Microbiology Institute
Portland (Wilsonville), OR

Judith Lovchik, Ph.D.
University of Maryland Medical System
Baltimore, MD

Chris N. Mangal, MPH
Association of Public Health Laboratories

Washington, DC

Susan E. Sharp, Ph.D.
Kaiser Permanente
Portland, OR

James W. Snyder, Ph.D.
University of Louisville
Louisville, KY

David Welch, Ph.D.
Laboratory Corporation of America
Dallas, TX

Mary K. York, Ph.D.
MKY Microbiology Consultants
Walnut Creek, CA

Table of Contents: Botulinum toxin

- I. General Information
 - A. Description of Organism
 - B. History
 - C. Geographic Distribution
 - D. Clinical Presentation

- II. Procedure
 - A. General
 - B. Precautions
 - C. Specimens
 - 1. Acceptable specimens
 - a. Feces
 - b. Enema
 - c. Gastric aspirate or vomitus
 - d. Serum
 - e. Tissue or exudates
 - f. Postmortem
 - g. Food specimens
 - h. Swab samples (environmental or clinical)
 - i. Environmental samples
 - (i) Soil
 - (ii) Water
 - 2. Specimen handling
 - 3. Rejection criteria
 - D. Materials
 - 1. Supplies
 - E. Shipping
 - F. Limitations

- III. References

- IV. Appendices
 - A. Suggested specimens based on form of botulism
 - B. Specimen-related information

I. GENERAL INFORMATION

A. Description of organism

Clostridium botulinum is 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. *C. 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. 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.

B. History

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.

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 (see Suggested Reading).

C. 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.

D. Clinical presentation

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, 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. Botulism needs to be differentiated from other neurological diseases, including Landry-Guillain-Barre syndrome, tick paralysis, myasthenia gravis, and Lambert-Eaton syndrome. Once a presumptive clinical diagnosis is made, an intense epidemiologic investigation should ensue to identify other related cases.

II. PROCEDURE

A. General. Laboratory Response Network (LRN) Level A (Sentinel) laboratory procedures are designed to ensure the proper collection and distribution of appropriate specimens to designated testing laboratories.

B. Precautions

- 1. 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. Submit specimens without delay.**
- 2. DO NOT** attempt to culture, identify the organism, or attempt to perform toxin analysis.
- 3.** Sentinel laboratories should not accept environmental or animal specimens; such specimens should be forwarded directly to the state health laboratory.

C. Specimen

- 1. Acceptable specimens** (for testing at Level C [LRN Reference] laboratories)
 - a. Feces.** Place into sterile unbreakable container and label carefully. Confirmatory evidence of botulism may be obtained from 10- to 50-g quantities (walnut size); botulism has been confirmed in infants with only "pea-size" stool samples.
 - b. Enema.** Place approximately 20 ml into a sterile unbreakable container and label carefully. If an enema must be 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.
 - c. Gastric aspirate or vomitus.** Place approximately 20 ml into a sterile unbreakable container and label carefully.
 - d. Serum.** Use red top or serum separator tubes to obtain serum (no anticoagulant). Samples should be obtained as soon as possible after the onset of symptoms and before antitoxin is given. Enough blood should be collected to provide at least 10 ml of serum for mouse toxicity tests (usually 20 ml of whole blood); serum volumes less than 3 ml will provide inconclusive results. Whole blood should not be sent, because it typically undergoes excessive hemolysis during transit.

- e. **Tissue or exudates.** Place into sterile unbreakable container and label carefully. Specimens should be placed in anaerobic transport media and sent to the appropriate laboratory for attempted isolation of *C. botulinum*.
- f. **Postmortem.** Obtain specimens of intestinal contents from different levels of small and large intestines. Place approximately 10 g per specimen into a sterile unbreakable container and label carefully. Obtain gastric content, serum, and tissue specimens if or as appropriate.
- g. **Food specimens.** Foods should be left in their original containers if possible, or placed in sterile unbreakable containers and labeled carefully. Place containers individually in leakproof containers (e.g., sealed plastic bags) to prevent cross-contamination during shipment. Empty containers with remnants of suspected foods can be examined.
- h. **Swab samples (environmental or clinical).** Send clinical swabs in an anaerobic transport medium. Environmental swabs (from which spores may be isolated) may be sent in plastic containers without any medium. Swabs may be shipped at room temperature or refrigerated. Collect three to four swabs from each potential site.
NOTE: Sentinel laboratories should not accept environmental (or animal) specimens: such specimens should be directly forwarded to the appropriate LRN Reference laboratory.
- i. **Environmental samples.** Collect a sample in the size indicated below for each possible location.
 - (i) Soil (50 to 100 g)
 - (ii) Water (~100 ml)**NOTE: Sentinel laboratories should not accept environmental (or animal) specimens. Such specimens should be directly forwarded to the appropriate LRN Reference laboratory.**

2. Specimen handling

- a. Store all specimens at 4°C and ship on cold packs as soon as possible.
- b. Submit to a LRN Reference laboratory as soon as possible.

3. Rejection criteria

- a. **Incomplete documentation.** All specimens must include the sender's name and telephone number to contact for the preliminary report and additional information.
- b. **Improper packaging/shipping**
- c. **Lack of prior approval.** Do not ship specimens to higher-level LRN laboratories without prior approval.

D. Materials

1. Supplies

- a. **Anaerobic transport vials**
- b. **Leakproof containers** (i.e., sealed plastic bags and other plastic containers)
- c. **Packaging materials** (Refer to ASM procedure on Transportation and Transfer of Biological Agents <http://www.asm.org/Policy/index.asp?bid=6342>)

E. Shipping. Refer to ASM procedure on Transportation and Transfer of Biological Agents <http://www.asm.org/Policy/index.asp?bid=6342>. Complete and attach appropriate documentation.

F. Submitting samples to LRN Reference laboratory

1. Submit samples to a laboratory approved to perform testing as directed by the state public health laboratory. Note that not all states currently have testing capability.
2. Toxin testing is performed primarily by a mouse toxicity and toxin neutralization method. Final results may be expected after 3 days.

G. Limitations. If the patient has been taking 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.

III. REFERENCES

1. **Allen, S. D., C. L. Emery, and D. M. Lyerly.** 2003. *Clostridium*, p. 835-856. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 8th 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, 23^{AL}, 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 Nations' Special Commission (UNSCOM). United Nations, New York, N.Y.
9. **Hatheway, C. L.** 1995. Botulism: the present status of the disease. *Curr. Top. Microbiol. Immunol.* **195**:55-75.

10. **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.
11. **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.
12. **Merson, M. H., and V. R. Dowell, Jr.** 1973. Epidemiologic, clinical and laboratory aspects of wound botulism. *N. Engl. J. Med.* **289**:1105-1110.
13. **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.
14. **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.
15. **St. Louis, M. E., S. H. Peck, D. Bowering, G. B. Morgan, J. Blatherwick, S. Banerjee, G. D. Kettlys, 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.
16. **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.
17. **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.
18. **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

1. **Infectious Diseases Society of America.** 2002. Bioterrorism information and resources. <http://www.idsociety.org>.
2. **Infectious Diseases Society of America.** Botulism, 20 February 2003. http://www.idsociety.org/bt/biotemplate.cfm?template=bot_summary.htm.

IV. APPENDICES

Appendix A. Suggested specimens based on form of botulism

1. Foodborne

- a. **Clinical material.** Serum, gastric contents, vomitus, stool, return from sterile water enema or saline enema
- b. **Autopsy samples.** Intestinal contents and gastric contents (serum if available)
- c. **Food samples**

2. Infant

- a. **Feces**
- b. **Return from sterile water or sterile saline enema**
- c. **Serum.** Although circulating toxin may be detected in infants with botulism, it is rare. Shipment of other specimens should not be delayed while waiting for serum collection.
- d. **Postmortem samples.** Intestinal contents from different levels of small and large intestine
- e. **Food and environmental samples** (as appropriate for the investigation)

3. Wound

- a. **Serum**
- b. **Exudate, tissue, or swab samples of wound transported in an anaerobic transport medium**
- c. **Feces or return from sterile water enema** (wound may not be source)
- d. **An isolate of suspected *C. botulinum*** (maintain under anaerobic conditions)

4. Intentional toxin release (inhalational or ingested)

- a. **Serum**
- b. **Feces or return from sterile water enema**
- c. **Food, solid or liquid**
- d. **Environmental or nasal swabs**
- e. **Gastric aspirate**

Appendix B. Specimen-related information

1. Food

- a. Foods most likely to allow growth of *C. botulinum* will have a pH range of 3.5 to 7.0; the most common pH is 5.5 to 6.5. However, suspected foods, regardless of pH, can be examined, since localized environmental conditions may be present that may support the growth of *C. botulinum*.
- b. Botulinum toxin in commercial products is rare. The state public health laboratory should notify the FDA at (301) 443-1240 if a commercial product is suspected of containing botulinum toxin.

2. **Feces.** *C. botulinum* has been isolated from stools following antitoxin treatment.