

Level A Laboratory Guidelines for Identification of *Clostridium botulinum* Toxin

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

II. Precautions: Refer to Procedure for Laboratory Safety and Decontamination.

III. Specimen

A. Acceptable specimens

1. Clinical specimens
2. Postmortem specimens
3. Culture/isolate
4. Food samples, solid or liquid
5. Environmental samples

B. Rejection criteria

1. Incomplete documentation: All specimens must include the sender's name and telephone number to contact for the preliminary report and additional information.
2. Improper packaging/shipping
3. Do not ship specimens to higher-level LRN laboratories without prior approval.

IV. Materials

A. Media: Anaerobic media (chopped meat or equivalent); follow standard laboratory procedures.

B. Supplies

1. Port-A-Cul vials (Becton Dickinson; catalog #4321609) or equivalent
2. Leakproof containers (i.e., sealed plastic bags, and other plastic containers)
3. Petroleum jelly, or petrolatum (Fisher Scientific; catalog #P661LB), or equivalent (e.g., Vaseline)
4. Sterile, nonbacteriostatic water
5. Packaging materials: Refer to Shipping Procedure.

Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources; inclusion does not imply endorsement by the Centers for Disease Control and Prevention, the Department of Health and Human Services, or the Federal Bureau of Investigation.

V. Quality control: Use standard laboratory criteria and procedures.

VI. Procedure

A. Collection: Collect samples according to established laboratory procedures.

1. Feces: Place into sterile unbreakable container and label carefully. Confirmatory

evidence of botulism may be obtained from 10-50 g quantities (English walnut size); botulism has been confirmed in infants with only "pea-sized" stool samples.

2. Enema: Place ~20 ml into 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.
3. Gastric aspirate or vomitus: Place ~20 ml into sterile unbreakable container and label carefully.
4. Serum: Use red top or separator type 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 whole blood); serum volumes less than 3 ml will provide inconclusive results. Whole blood should not be sent as it typically undergoes excessive hemolysis during transit.
5. Tissue or exudates: Place into sterile unbreakable container and label carefully. Specimens should be placed in Port-A-Cul vials (Mena, 1978) and sent to the appropriate laboratory, preferably without refrigeration, for attempted isolation of *C. botulinum*.
6. Postmortem: Obtain specimens of intestinal contents from different levels of small and large intestines. Place ~10 g per specimen into sterile unbreakable container and label carefully. Obtain gastric content, serum, and tissue specimens if/as appropriate (refer to A. 3, 4, and 5 above).
7. Culture: Ship suspicious isolates anaerobically (overlay liquid media with 2-inch sterile petroleum jelly or petrolatum; melt/temper prior to overlaying culture). Cultures may be shipped at room temperature or refrigerated.
8. 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 (i.e., sealed plastic bags) to prevent cross-contamination during shipment. Empty containers with remnants of suspected foods can be examined.
9. Swab samples (environmental or clinical): Send clinical swabs in an anaerobic transport medium (e.g., Port-A-Cul tubes) (Mena, 1978). 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 3-4 swabs from each potential site.
10. Environmental samples: Collect a sample in the size indicated below for each possible location.
 - (1) Soil (50-100 g)
 - (2) Water (\geq 100 ml)

B. Shipping: Refer to Shipping Procedure; complete and attach appropriate documentation.

1. Specimens sent to a distant laboratory should be placed in sterile leakproof containers, then in insulated shipping containers with refrigerant (sealed ice packs, cold packs), labeled "MEDICAL EMERGENCY, BIOLOGICAL HAZARD, REFRIGERATE ON ARRIVAL" and should be shipped by the most rapid means available. Most of the major airlines have a special package handling service for expedited shipments. Do not send by U.S. Postal Service; ship as hazardous materials.

2. If an unavoidable delay of several days is anticipated, the specimens (serum or stool) should be kept frozen and then packed in an insulated container with dry ice and proper cushioning material for shipment. Freezing does not significantly affect the stability of botulinum toxin in specimens; freezing does reduce the probability of recovering *C. botulinum*. Since direct detection of toxin provides the best laboratory confirmation of botulism, priority should be given to preserving preformed toxin prior to transport.
3. The receiving laboratory should be notified in advance by telephone as to when and how specimens will be shipped, and when they will arrive.
4. Forward patient history and clinical symptom information to reference laboratory with the specimens.

VII. Reporting/action

A. Consult with state public health laboratory director (or designate) if *C. botulinum* toxin is suspected.

B. General instruction and information

1. Preserve original specimens pursuant to a potential criminal investigation and possible transfer to an appropriate LRN laboratory as instructed.
2. Environmental/nonclinical samples and samples from announced events should not be received by a Level A laboratory; submitter should contact the state public health laboratory directly.
3. The state public health laboratory/state public health department will coordinate notification of local FBI agents as appropriate.
4. Assist local law enforcement efforts in conjunction with guidance received from the state public health laboratory.
5. FBI and state public health laboratory/state public health department will coordinate the transfer of isolates/specimens to a higher level LRN laboratory as appropriate.

C. In conjunction with state public health laboratory, the laboratory may contact CDC as appropriate.

1. Emergency number, 24 h a day, 7 days a week: 770-488-7100
2. National Botulism Surveillance and Reference Laboratory: 404-639-3867

VIII. Limitations

A. 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 (Horwitz, 1976).

B. Recovery of viable cells from specimens often proves difficult. Proper handling, packaging, and shipping with minimal delay improves probability of recovery.

IX. Procedure notes

A. Suggested specimens based on form of botulism

1. Foodborne
 - a. Clinical material: Serum, gastric contents, vomitus, stool, return from sterile water 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 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

B. Specimen-related information

1. Food
 - a. Foods most likely to allow growth of *C. botulinum* will have a pH range of 3.5-7.0, the most common pH is 5.5-6.5. 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.