UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
ENVIRONMENT, HEALTH AND SAFETY/BIOSAFETY

BOTULINUM TOXIN EXPOSURE/INJURY RESPONSE PROTOCOL

Organism or Agent: Botulinum Toxin produced by Clostridia species
Exposure Risk: Botulism
UCSF Occupational Health Services: 415/885-7580 (Available during work hours)
Exposure Hotline: 415/353-7842 (Available 24 hours)
Office of Environment, Health & Safety: 415/476-1300 (Main number; work hours)
415/476-1414 or 9-911 (In case of emergency, via the UCSF Police Department; available 24 hours)
Public Health Officer: 415/514-3531
Biosafety Officer (Responsible Official): 415/514-2824

PROTOCOL SUMMARY

In the event of an accidental exposure or injury, the protocol is as follows:

1. Modes of Exposure:
   a. Skin puncture or injection
   b. Ingestion
   c. Contact with mucous membranes (eyes, nose, mouth)
   d. Contact with non-intact skin
   e. Respiratory exposure from inhalation of toxin

2. First Aid:
   a. **Skin Exposure:** Immediately go to the sink and thoroughly wash the skin with soap and water.
   b. **Skin Wound:** Immediately go to the sink and thoroughly wash the wound with soap and water and pat dry.
   c. **Splash to Eye(s), Nose or Mouth,** immediately flush the area with running water for at least 15 minutes.
   d. **Inhalation Exposure:** Report to the Emergency Department for medical treatment immediately.
   e. **Injection or Needlestick Exposure:** Report to the Emergency Department for medical treatment immediately.
   f. **Splash Affecting Garments:** Remove all soiled or contaminated garments and double-bag them in red biohazard plastic bags.

3. Treatment:
   In the event of an acute injury or exposure resulting from a laboratory incident, the injured individual should report immediately to the Emergency Department for medical treatment, especially for inhalation and injection exposures. The injured individual must take a copy of this entire protocol document to the Emergency Department, including information regarding the specific toxin subtypes associated with exposure. The HBAT botulism antitoxin is available in the United States for treatment of botulism and is available through the California Department of Public Health and the CDC. See Appendix A, item E: “Post-exposure Prophylaxis/Treatment” for detailed information.

4. Follow-up is needed in the event of any Laboratory Exposure:
   a. After first aid has been administered, immediately inform your supervisor about the exposure.
   b. In the event of a large spill in a secure area, leave the area and secure the lab to prevent entry of other personnel and possible secondary exposures. In the event of a spill in a non-secure area, contact the EH&S Emergency Response Team (9-911) for clean-up.
   c. Contact the Exposure Hotline to report a laboratory exposure to botulinum toxin.
   d. Report to Biosafety officer (Responsible Official) Peili Zhu at 514-2824 immediately.
1. **WORKER’S RESPONSIBILITIES (Employee/Student Initial Self-Care)**
   a. **First Aid:** Perform recommended first aid and decontamination according to the posted instructions.
      
      **Treatment:**
      i) In the event of an acute exposure or injury, injured individual must report to the Emergency Department for acute medical treatment. The employee shall provide the following information to the physician: the specific botulinum toxin subtype associated with exposure; the quantitative exposure amount and mode of exposure; and the signs and symptoms of exposure that the employee is experiencing.
      ii) In the event of an exposure, with or without injury, call the Exposure Hotline at 415/353-7842.
   b. **Reporting:** Inform your laboratory supervisor/principal investigator of the exposure.
   c. **Secure the laboratory:** Identify the equipment involved in the exposure and the mechanism of exposure. Make sure that the laboratory area has been secured and that notification of contamination has been posted to prevent other individuals from entering the area.

2. **SUPERVISOR’S RESPONSIBILITIES**
   a. **First Aid and Decontamination:** Verify that the worker has washed and decontaminated himself/herself. Confirm that if there was an injury, the employee went to the Emergency Department immediately and that the employee has contacted the Needlestick Exposure Hotline.
   b. **Secure the laboratory:** Confirm that the laboratory area has been secured and that notification of contamination has been posted to prevent other individuals from entering the area.
   c. **Laboratory clean-up (as needed):** Contact the Office of Environment, Health & Safety (OEH&S) through the UC Police Department Emergency Dispatch (from a campus telephone 9-911, from a non-campus phone 415/476-1414).
   d. **Report the exposure:** Call the Biosafety Officer (Responsible Official) Peili Zhu at 415/514-2824 immediately and the Public Health Officer to report the exposure.
   e. **Follow Up:** Confirm that the worker has called for an appointment at the UCSF Occupational Health Clinic.
   f. **Report the Injury:** Within 24 hours, report the injury to the UCSF Human Resources Disability Management Services (HR DMS) Office on the Supervisor Incident From (SIR) form, available here: [http://ucsfhr.ucsf.edu/files/SIR.pdf](http://ucsfhr.ucsf.edu/files/SIR.pdf)
UNIVERSITY OF CALIFORNIA SAN FRANCISCO
ENVIRONMENT, HEALTH AND SAFETY
TOXIN DATA SHEET
BOTULINUM TOXIN
FOR USE IN RESEARCH LABORATORIES

MATERIAL SAFETY DATA SHEET – TOXIC SUBSTANCES

SECTION I – Toxin
Organism or Agent: Botulinum Neurotoxin – produced by Clostridia species
Synonym or Cross Reference: Botulism toxin
Characteristics: Botulinum Neurotoxin (BoNT) is a toxin produced by the gram positive rod shaped bacteria, Clostridium botulinum. Botulinum neurotoxin is one of the most toxic substances known and is active orally, inhalationally, and parenterally. It causes the human disease botulism when ingested or inhaled. The agent is toxic and in sufficient amounts has a potential for high socio-economic and/or public health impact. Botulinum toxin is defined as a Tier 1 Select Agent by the CDC and USDA.

SECTION II - Health Hazard
Pathogenicity: Rare but serious paralytic disease, caused by a neurotoxin formed during the growth of the spore-forming bacterium C. botulinum (or rarely, C. argentinense, C. butyricum, or C. baratti ). This neurotoxin binds to the neuromuscular junction and blocks excitatory synaptic transmission by inhibiting acetylcholine release, causing (flaccid) paralysis, and sometimes fatal respiratory failure. The fatality rate of botulism is 5 to 10%.
Botulinum neurotoxin is not an infectious agent, however all lab personnel and particularly women of child-bearing age should be aware that it is possible that immune compromised individuals may be predisposed to intoxication. Individuals who have conditions that may compromise immune response are encouraged to identify themselves to the UCSF Employee and Health Services Department at 415-885-7580 for appropriate counseling and guidance.
Acute Toxicity: The estimated lethal oral dose is 70 micrograms in a 70-kilogram human (154lbs). The lethal intramuscular dose is 0.09 to 0.15 micrograms. The lethal inhalational dose of dry powder is 0.70 to 0.90 micrograms. Botulinum toxin becomes ~100X more lethal when inhaled, and ~1000X more lethal when introduced directly into the bloodstream.
Modes of Exposure:
1. Skin puncture or injection
2. Ingestion
3. Contact with mucous membranes (eyes, nose, mouth)
4. Contact with non-intact skin
5. Respiratory exposure from inhalation of toxin

Clinical Signs after Exposure: Clinical signs can usually be seen within several hours and include nausea, dizziness, blurred vision, difficulty swallowing, constipation/diarrhea, muscle weakness and difficulty breathing.

SECTION III - Laboratory Hazards
Laboratory-Acquired Infections: There has been only one report of botulism associated with handling of the toxin in a laboratory setting.
Primary Hazards: Exposure to the toxin by inhalation of toxin, spilling of solution on broken skin, splashing of solution in the eye, or injury by contaminated material as a cut from a broken glass container that had contained toxin solution.
SECTION IV - Recommended Precautions

Containment Requirements: Biosafety level 2 with materials known to contain or potentially containing the toxin; additional requirements are outlined in the lab-specific BUA.

Protective Clothing: Laboratory coat and gloves when handling toxin. Eye protection must be used where there is a known or potential risk of exposure to splashes.

Other Precautions: The use of needles, syringes, and other sharp objects should be strictly limited.

SECTION V - Handling Information

Spills: Allow aerosols to settle and while wearing protective clothing, gently cover the spill with paper towels and apply appropriate disinfectant starting at the perimeter, working inwards towards the center. Use solutions of sodium hypochlorite (0.5%) or sodium hydroxide (0.1M) to decontaminate spills for 30 minutes. Discard all waste in a red biohazard waste bag.

Disposal: Decontaminate before disposal; steam sterilization, incineration, chemical disinfection (sodium hydroxide). After decontamination, toxin waste will be collected for pick up and disposal as chemical waste by EH&S.

Storage: Toxin must be kept in secured location in sealed containers that are appropriately labeled. Lab keeps an inventory of toxin usage.

FOR USE BY THE EXPOSURE HOTLINE RESPONDER

SECTION VI - Viability

Susceptibility to Disinfectants: Solutions of sodium hypochlorite (0.5%) or sodium hydroxide (0.1M) readily inactivate the toxin and are recommended for decontamination of work surfaces and for spills. Autoclaving of contaminated materials also is appropriate.

SECTION VII - Medical

Surveillance: Monitor for symptoms; demonstration of toxin in serum, stool, gastric aspirate or implicated food

First Aid/Treatment: Intravenous/intramuscular administration of trivalent (ABE) or heptavalent (A,B,C,D,E,F,G) botulinum antitoxin; assisted ventilation if respiratory failure occurs.

Immunization: None

Red Flags: Symptoms of toxin exposure typically begin with difficulty seeing, speaking and/or swallowing. Other symptoms may include fatigue, dizziness, double vision, blurred vision, problems swallowing, dry mouth, difficulty speaking, sore throat, shortness of breath, constipation, nausea vomiting, abdominal cramps, diarrhea, arm or leg weakness, and paresthesias. Onset of any of these symptoms indicates the need for immediate treatment in an Emergency Department.

Post Exposure Prophylaxis: An equine antitoxin product (H-BAT) is available for treatment of patients with symptoms consistent with botulism. However, due to the risks of inherent in equine products, treatment is not provided as a result of exposure unless botulism symptoms are present. This antitoxin is only available through the California State Department of Public Health and CDC. Please see appendix A, item E “Post-exposure Prophylaxis/Treatment” for detailed information.
SECTION IX – Emergency Medical Treatment

Treatment Indications: Onset of symptoms may occur 2-72 hours after exposure. Treatment with antitoxin may be considered in the absence of symptoms in case of an especially severe or large exposure. Lethal doses in a 70 kilogram person are estimated to be 0.09-0.15μg intravenously, 0.70-0.90μg inhalationally, and 70μg orally. BoNT becomes ~100X more lethal when inhaled, and ~1000X more lethal when introduced directly into the bloodstream. In the event that an exposure significantly above the amount used medically (an order of magnitude or greater than standard Botox/Myoblock doses), or development of symptoms indicative of poisoning, emergent treatment with antitoxin should be considered. Disease symptoms are similar regardless of type of botulism toxin.

Treatment: An equine antitoxin product (HBAT) is available for treatment of patients with symptoms consistent with botulism. However, due to the risks of inherent in equine products, treatment is not provided as a result of exposure unless botulism symptoms are present. This antitoxin is only available through the California State Department of Public Health and CDC. Please see appendix A, item E “Post-exposure Prophylaxis/Treatment” for detailed information.

Investigation: The emergency room must immediately call the San Francisco County Department of Public Health Disease Control Unit at (415) 554 – 2830 to report the exposure and follow any specific instructions on specimen collection. During off hours, press 1 and 1 again after calling the number above to page the on-call physician.

Monitoring: Sequential spirometry is recommended as a means of assessing for potential respiratory muscular weakness. Sequential assessment of FVC and inspiratory force may provide early clues of impending respiratory failure.

A careful neurologic examination will also be helpful in following potentially exposed patients. Note especially cranial nerves and reflexes. Paralysis always begins in bulbar musculature. It is not possible to have botulism without multiple cranial nerve palsies.

All patients with a history of botulinum exposure who are symptomatic (e.g. diplopia, ptosis, dysphagia, sore throat, muscle weakness) should be admitted to an intensive care unit because of the risk of sudden respiratory compromise.

Discharge / Follow-up:

Any patient seen in the Emergency Department and released should be given information about the potential for delayed onset of symptoms/toxicity. Any symptoms would be reason for emergency reevaluation. Typical symptoms include difficulty seeing, speaking, and/or swallowing. All exposed individuals should also be referred to the UCSF Occupational Health Services for follow-up care.

The Emergency Department physician shall ensure that the Needlestick Exposure Hotline has been notified about the exposure.
References


Investigational heptavalent botulinum antitoxin (HBAT) to replace licensed botulinum antitoxin AB and investigation botulinum antitoxin E. 2010. Center for Disease Control. MMWR 59 (100:299).
APPENDIX A

I. RISKS IN LABORATORY WORKERS/CLINICAL SUMMARY

A. Overview

Clostridium botulinum and rare strains of C. baratii and C. butyricum are anaerobic spore-forming species that cause botulism, a life-threatening food-borne illness. The pathogenicity of these organisms result from the production of botulinum toxin, one of the highly potent neurotoxins currently recognized. Purified botulinum neurotoxin is a 150kDa protein that acts selectively on peripheral cholinergic nerve endings to block neurotransmitter release. The principal site of action is the neuromuscular junction, where blockade of transmission produces muscle weakness or paralysis. The toxin also acts on autonomic nerve endings where blockade of transmission can produce a variety of adverse effects. The toxin may also contain associated proteins that may increase its size to as high as 900kDa.

Occupational Infections
There has been only one report of botulism associated with handling of the toxin in a laboratory setting. However, concerns about potential use of the toxin as an agent of bioterrorism or biological warfare have led to increased handling of the substance by investigators studying mechanism of action and/or developing countermeasures to poisoning.

Laboratory Safety and Containment Recommendations
Neurotoxin producing Clostridia species or its toxin may be present in a variety of food products, clinical materials (serum, feces) and environmental samples (soil, surface water). In addition, bacterial cultures may produce very high levels of toxin. In healthy adults, it is typically the toxin and not the organism that causes disease. Risk of laboratory exposure is due to the presence of the toxin and not due to a potential of infection from the organisms that produce the toxin. Although spore-forming, there is no known risk to spore exposure except for the potential for the presence of residual toxin associated with pure spore preparations. Laboratory safety protocols should be developed with the focus on prevention of accidental exposure to the toxin produced by these Clostridia species.

The above section is taken directly from the BMBL 5th Edition: http://www.cdc.gov/biosafety/publications/bmbl5/index.htm

B. Toxin Subtypes:

Types A and E neurotoxins “bind specifically to the presynaptic membrane of cholinergic neurons and penetrate into the cytosol where they cleave specifically SNAP-25. This selective proteolysis impairs the functional participation of SNAP-25 in the neuroexocytosis machine and the release of acetylcholine evoked by membrane depolarization cannot take place.”¹

Types B, D, F, and G neurotoxins “cleave VAMP/synaptobrevin, a protein of small synaptic vesicles. This results in loss of function of the neuroexocytosis machinery and thus a blockade of transmitter release.”¹

Type C neurotoxin “binds specifically to nerve terminals and is then translocated to the cytosol where it cleaves syntaxin, and SNAP-25 presynaptic membrane proteins involved in the exocytotic machinery acting as a t-SNARE.”¹
C. Diagnostic Tests/Clinical Signs & Symptoms

Diagnostic testing is not useful in acute management, but blood serum should be obtained and held for later analysis. See section E Post-exposure prophylaxis/treatment on specimen collection.

Clinical signs and symptoms should guide clinical treatment. The clinical syndrome is distinctive, and generally consists of cranial nerve palsies may be followed by flaccid descending paralysis of voluntary muscles, affecting (in order) the muscles of the neck, shoulders, the proximal and then distal upper extremities, and the proximal followed by distal lower extremities. Cranial nerve palsies (are symmetrical), followed by descending flaccid paralysis that may progress to respiratory arrest; eventual constipation is a nearly universal symptom; vital signs are usually normal; in some cases hypotension occurs. Deep tendon reflexes progressively disappear. The ultimate extent of paralysis in untreated patients and the rapidity of progression are variable. Symptoms may progress over hours to days, with the rate apparently proportional to dose. Paralysis resolves in weeks to months, and often requires extended outpatient rehabilitation therapy. Intellectual function is preserved throughout. Tragically, in some instances, the patient’s ptosis, expressionless facies, and altered voice have been interpreted as signs of mental status changed due to alcohol intoxication, drug overdose, or meningitis.”

D. Pre-exposure Prophylaxis

Currently there is no vaccine available for immunization of laboratory personnel working with botulinum toxin or cultures of botulinum neurotoxin producing species of Clostridium. Although administered under an FDA IND since 1965, CDC discontinued distribution of the Pentavalent (ABCDE) Botulinum Toxoid vaccine in 2011 due to decline in potency and CDC observed increase in moderate local reactions. An equine-based heptavalent (A,B,C,D,E,F, and G) antitoxin is available through a CDC-sponsored FDA IND for treatment of individuals with symptoms consistent with botulism. Health-care providers for exposed laboratory personnel should consult their state health department epidemiologist to determine if use of HBAT is warranted.

E. Post-exposure Prophylaxis/Treatment

As of March 13 2010, the new heptavalent botulinum antitoxin, HBAT, became the only botulism antitoxin available in the United States for naturally occurring noninfant botulism. It is available only from the CDC because of its limited use and its relatively short expiration date. The antitoxin is released only for suspected or actual cases of botulinum toxin poisoning and works by neutralizing unbound toxin molecules. Medical care providers who suspect a diagnosis of botulism in a patient should immediately call their state health department's emergency 24-hour telephone number to maintain effective botulism surveillance and to detect outbreaks as soon as possible.

In California, the local county department of public health must be notified immediately of any suspect case by calling (415) 554 – 2830. Press 1 and 1 to page the on-call physician during off hours. The San Francisco County will conduct an initial assessment of the case and will contact California State Department if release of botulism antitoxin is needed. Additional emergency consultation is available from the CDC botulism duty officer via the CDC Emergency Operations Center telephone, (770) 488-7100.

F. Additional Background Information (from BMBL 5th Edition):

Select Agent: Neurotoxin-producing Clostridia species are Select Agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer.

Transfer of Agent: Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country.
G. Follow Up
Contact Occupational Health Services (415/885-7580) for follow up.

References:


Investigational heptavalent botulinum antitoxin (HBAT) to replace licensed botulinum antitoxin AB and investigation botulinum antitoxin E. 2010. Center for Disease Control. MMWR 59 (100:299).
