

Exposure Response Plan for Laboratory Handling of Botulinum toxin type A

Background Information

Botulinum toxin type A (BoNT/A) is a potent neurotoxin produced by the anaerobic, spore-forming bacterium *Clostridium botulinum*. It is one of seven immunologically distinct serotypes (A–G), with type A being among the most clinically relevant for both human disease and therapeutic use. BoNT/A exerts its effect by blocking the release of acetylcholine at the neuromuscular junction, resulting in localized, reversible muscle paralysis.

Although BoNT/A is utilized in extremely small, controlled doses for medical and cosmetic purposes (e.g., treatment of muscle spasticity, migraines, and dystonia), it remains a high-risk biological toxin due to its extreme potency. Even trace amounts can cause serious or fatal effects if mishandled or accidentally released. For this reason, BoNT/A is designated as a **Select Agent Toxin** under U.S. federal regulations (42 CFR Part 73) and must be handled under strict institutional biosafety controls.

In laboratory settings, work involving BoNT/A is limited to authorized personnel who have completed specific biosafety and toxin-handling training. Activities are typically performed within a certified biosafety cabinet using engineering controls, personal protective equipment (PPE), and clearly documented standard operating procedures (SOPs).

While BoNT/A does not spread person-to-person, exposure through inhalation, ingestion, or percutaneous routes can lead to botulism, characterized by descending flaccid paralysis, blurred vision, difficulty swallowing, and respiratory distress. Rapid recognition and immediate medical evaluation are essential, as prompt supportive care and antitoxin administration can significantly reduce morbidity and mortality.

This response plan provides procedures for **safe laboratory handling, spill containment, potential exposure response, and medical follow-up** related to BoNT/A, ensuring compliance with institutional biosafety requirements and public health regulations.

Host Range

Botulinum toxin-producing *Clostridium botulinum* affects a broad range of species. Natural hosts include humans, birds, cattle, fish, mink, foxes, cats, dogs, and pigs, while flies and their larvae may act as secondary hosts and environmental vectors.

Experimental infections have also been demonstrated in mice and non-human primates, which serve as valuable research models for understanding the pathogenesis, neurotoxicity, and clinical effects of the toxin.

Modes of Transmission

In the laboratory setting, transmission of botulinum toxin type A occurs only through direct exposure to the purified toxin or contaminated materials. The toxin is not infectious—it does not replicate in host tissues and cannot spread from person to person. Laboratory-associated intoxication may result from one or more of the following exposure routes:

- Inhalation: Aerosol generation during manipulation outside a certified biosafety cabinet (e.g., pipetting, vortexing, or centrifugation) can lead to inhalational exposure.
- Percutaneous: Accidental needle sticks, cuts, or skin punctures involving toxin-contaminated sharps or glassware.
- Ingestion: Hand-to-mouth contact or accidental ingestion of contaminated materials due to inadequate hygiene or failure to remove gloves properly.
- Mucous membrane contact: Splash or droplet exposure to the eyes, nose, or mouth during handling of liquid toxin preparations.

Strict adherence to engineering controls, standard operating procedures (SOPs), and personal protective equipment (PPE) requirements minimizes these risks. Work must always be performed within a certified biosafety cabinet (Class II or higher) to prevent aerosol release, and any manipulations involving concentrated toxin should follow enhanced BSL-2 practices (often termed BSL-2+).

Laboratory Hazards

The primary laboratory hazards associated with *Clostridium botulinum* and its neurotoxin (BoNT) include ingestion, contact with damaged skin or mucous membranes, and inhalation of aerosolized toxin particles. These exposure routes can result in systemic intoxication, emphasizing the need for stringent containment, use of personal protective equipment, and adherence to established biosafety practices.

Working with Animals Exposed to Botulinum Toxin Type A

Biosafety Level

- Animal studies involving BoNT/A must be conducted under Animal Biosafety Level 2 (ABSL-2) or enhanced ABSL-2 (ABSL-2+) conditions, depending on toxin concentration and procedure risk (e.g., injection, necropsy, aerosol potential).
- Procedures that may generate aerosols (e.g., toxin dilution, injection, necropsy) should be performed within a certified Class II biosafety cabinet (BSC).

Animal Handling and PPE

- Only trained and authorized personnel may handle animals treated with BoNT/A.

- Required PPE includes: lab coat or disposable gown, double nitrile gloves, eye/face protection, and respiratory protection (N95 or equivalent) if aerosol risk exists.
- Use bite-resistant gloves or restraint devices for animals that may exhibit neurological signs (e.g., weakness, paralysis).
- Perform all toxin preparation and administration inside the BSC; administer injections using safety-engineered sharps when possible.

Animal Monitoring and Clinical Signs

Animals receiving BoNT/A may develop clinical signs consistent with neuromuscular blockade:

- Decreased activity or limb weakness
- Ptosis (drooping eyelids)
- Difficulty swallowing or breathing
- Progressive, symmetrical paralysis
- Daily monitoring is required

Signs and Symptoms of Exposure

Exposure to botulinum toxin type A (BoNT/A) can lead to the clinical syndrome known as botulism, which results from the toxin's blockade of acetylcholine release at neuromuscular junctions. The onset of symptoms typically occurs 12 to 72 hours after exposure, depending on the route and dose.

Early symptoms may include:

- Blurred or double vision (diplopia)
- Drooping eyelids (ptosis)
- Difficulty speaking (dysarthria)
- Difficulty swallowing (dysphagia)
- Dry mouth and throat
- Generalized weakness or fatigue

Progressive symptoms can include:

- Symmetrical, descending flaccid paralysis (starting in cranial nerves and progressing to limbs)
- Respiratory muscle weakness or paralysis
- Diminished or absent deep tendon reflexes
- In severe cases, respiratory failure and death if untreated

Additional notes:

- Sensory function and mental status remain intact.
- There is no fever unless secondary infection occurs.
- Onset may be delayed following dermal or mucosal exposure.

Medical urgency:

Any suspected exposure or onset of neurologic symptoms should be treated as a medical emergency. Immediate evaluation, supportive care (including respiratory support), and prompt notification of public health authorities are required. Administration of botulinum antitoxin can prevent further progression if given early.

Incubation Period

The incubation period for botulinum toxin type A varies depending on the route, dose, and type of exposure:

- Ingestion or inhalation: Symptoms typically appear within 12 to 72 hours, but onset can range from a few hours to up to 10 days in some cases.
- Percutaneous or mucosal exposure: Onset may be delayed, often occurring several days after exposure, due to slower systemic absorption.
- Injection (iatrogenic exposure): Clinical effects can appear within hours to several days, depending on dose and distribution.

In all cases, the progression of symptoms is gradual and descending, emphasizing the need for early recognition and immediate medical intervention.

Medical Precautions/Treatments

Prophylaxis

There is no antibiotic or chemoprophylaxis available for prevention of botulinum toxin intoxication. Because the toxin is a non-infectious protein rather than a replicating organism, antibiotics are ineffective for preventing or treating intoxication except in wound botulism, where they target *C. botulinum* infection (typically penicillin or metronidazole). In laboratory settings, prevention relies entirely on strict adherence to biosafety practices, including containment, PPE, and engineering controls to avoid exposure.

Vaccines

A pentavalent botulinum toxoid vaccine (types A–E) has been developed for laboratory and military personnel at elevated risk; however, it is not widely available and is considered investigational under the U.S. FDA’s Expanded Access program. Routine vaccination is not generally recommended unless work involves large quantities or frequent manipulations of botulinum toxin or cultures.

Medical Treatments and Antitoxin Access (California)

Treatment Overview

There is no antidote that reverses the effects of botulinum toxin once it has bound to nerve terminals. Management focuses on supportive care—particularly respiratory support—and administration of botulinum antitoxin to neutralize any circulating,

unbound toxin. Early recognition and rapid treatment are critical to prevent further progression of paralysis.

- Supportive care: Maintain airway, monitor respiratory function, and provide mechanical ventilation if respiratory compromise occurs.
- Antitoxin therapy: The Heptavalent Botulinum Antitoxin (HBAT), derived from equine antibodies, neutralizes toxin types A through G and is the standard treatment for adults and children in the United States.
- Timing: Antitoxin is most effective when administered as soon as possible after symptom onset or suspected exposure.
- Additional care: Hospitalization and continuous monitoring for several days to weeks may be required depending on severity.

Obtaining Antitoxin in California

Antitoxin for suspected botulinum toxin exposure is not stocked commercially and must be obtained through public health channels:

1. Contact the Local Health Department (LHD):
 - In California, the LHD is the first point of contact for healthcare providers and laboratories reporting a suspected exposure or intoxication.
 - The LHD coordinates directly with the California Department of Public Health (CDPH) to request antitoxin.
2. California Department of Public Health (CDPH):
 - CDPH authorizes and facilitates release of HBAT for adults and older children through coordination with the Centers for Disease Control and Prevention (CDC).
3. Centers for Disease Control and Prevention (CDC):
 - The CDC maintains the national stockpile of HBAT and dispatches the product through its Emergency Operations Center (EOC) once the state request is approved.
 - **The EOC can be reached 24/7 at 770-488-7100 for urgent clinical consultation and antitoxin release coordination.**

Key Contacts (California):

- **Local Health Department:** Contact your county health department's on-call officer (available 24/7).
- **California Department of Public Health (CDPH):** Division of Communicable Disease Control – www.cdph.ca.gov
- **CDC Emergency Operations Center:** 770-488-7100 (for public health officials only; coordination required via CDPH).

Post-Treatment Monitoring

Following exposure or treatment:

- Continue medical surveillance for **at least 10 days**, as delayed onset is possible.
- Notify Occupational Health and institutional biosafety personnel.
- Document exposure, treatment, and recovery outcomes per institutional protocols and report to CDPH/CDC if required.

Personal Protective Equipment (PPE)

Lab coat, gloves, closed-toed shoes, eye protection are required. Wash hands with soap and water after removing gloves.

Immediate Response Following Exposure

Mucous membrane

Eyes, mouth, nose, exposure: Flush eyes for 10-15 minutes with clean water, rinse mouth thoroughly without swallowing.

For any area not protected by skin, wash with soap and water for 15 minutes (open wounds, sores, etc.)

Reporting Exposure Incidents

1. Stop working immediately and alert nearby personnel.
2. Secure the area to prevent others from entering until it can be assessed by the Biosafety Officer or EH&S representative.
3. Remove contaminated PPE carefully to avoid secondary contact.
 - Place all disposable materials (gloves, gowns, wipes) in a biohazard bag for decontamination or disposal.
4. Decontaminate exposed skin immediately by washing thoroughly with soap and copious amounts of water.
 - For mucous membrane exposure (eyes, mouth, nose), flush with running water or sterile saline for at least 15 minutes.
5. Report the incident to the:

- **PI or laboratory supervisor**
- **UCR Biosafety Officer (BSO) and EHSRM at (951) 827-5528.**
- **Occupational Health ehsocchealth@ucr.edu**
- **You may contact the UCI Medical Center Infectious Disease Fellow on call at 714-456-6011 for immediate counseling and guidance. UCR maintains an agreement with the UCI Center for Occupational and Environmental Health (COEH) Clinic, which serves as our Occupational Health provider and reviews UCR's Animal Occupational Health Program.**

Undergraduate Student Employees report your injury to your supervisor (or go to [Employee Injuries](#)).

For life-threatening injuries, call 911 immediately.

For all other injury types, seek Medical Treatment at UCR's preferred Occupational Clinics. Visit the [Medical Treatment Facilities](#) webpage to learn more about where to seek medical treatment.

Seek medical evaluation even if symptoms are not present—onset may be delayed up to several days.

Stability

Disinfection

- 10% sodium hypochlorite (bleach) — minimum contact time of 10 minutes.
- 0.5% glutaraldehyde — effective for surface and equipment decontamination.
- 70% ethanol or isopropanol — suitable for small surface areas and follow-up cleaning after bleach application.

Surfaces should first be cleaned to remove organic material, as it can reduce disinfectant efficacy.

Inactivation

The toxin can be inactivated by moist heat at 56°C for 30 minutes or by boiling for 10 minutes.

Dry heat ($\geq 100^{\circ}\text{C}$) for ≥ 1 hour is also effective. The toxin is a protein and is rapidly denatured by high temperatures, extremes of pH (<4 or >10), and strong oxidizing agents.

Survival Outside Host

Although the toxin is relatively labile under standard environmental conditions, it may remain active for variable periods depending on the medium:

- Aqueous solutions (neutral pH, refrigerated): up to several weeks.
- Dried residues or contaminated surfaces: stable for days under cool, dark, and protein-rich conditions.
- Frozen or lyophilized preparations: may remain potent for months if not properly inactivated.

Therefore, all contaminated materials should be handled as biohazardous waste and decontaminated immediately after use.

References

- *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition — CDC / NIH biosafety guideline document <https://www.cdc.gov/labs/bmbl/index.html?utm>
- *Pathogen Safety Data Sheets: Clostridium botulinum (Canada)* — Public Health Agency of Canada <https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/clostridium-botulinum.html>

	University of California, Riverside INFECTIOUS AGENT CARD	
My job requires me to work with Botulinum toxin type A If the person with this card exhibits any of the symptoms listed on the back, immediately contact the UCI Medical Center Infectious Disease Fellow on call at (714) 456-6011. For immediate medical counseling on what do right away, proceed to the nearest Emergency Department and present this card. For more information or to report an incident, call UC Riverside Occupational Health 951-827-5528 (8- 4:30 M-F).		
Botulinum toxin type A is a potent neurotoxin that poses a significant risk of intoxication through accidental needle stick, aerosol inhalation, mucosal contact, or ingestion during laboratory or animal handling activities. <u>INCUBATION PERIOD:</u> 12–72 hours <u>SYMPTOMS:</u> Blurred vision, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, descending paralysis, respiratory distress <u>TREATMENT:</u> Supportive care + Heptavalent Botulinum Antitoxin (HBAT®); antibiotics (penicillin / metronidazole) only for wound cases		

Acknowledgement of Working with Botulinum toxin type A

By signing below, I confirm that I have reviewed and understood the requirements for working with Botulinum toxin type A. I agree to comply with all outlined responsibilities, including:

- Following safe laboratory practices and use of appropriate PPE
- Applying proper first aid and decontamination procedures in the event of an exposure
- Promptly reporting any exposures, incidents, or safety concerns to my supervisor, Biosafety Officer, and Occupational Health

Name (Print)	Identification*	Signature	Date	Supervisor / Principal Investigator

*Identification: Provide your UCR Student ID, Employee ID, UCR NetID, UCR Email, or Date of Birth.