



Warning

PI Name:

Safety Protocol for working with Pertussis Toxin

Hazard Communication Statement

Biological toxins are toxic substances that can be produced by bacteria, fungi, protozoa, insects, animals or plants and are classified separately from chemical toxins. They are nonreplicative, noninfectious materials but can be extremely hazardous, even in minute quantities. The toxicity and health hazard of biological toxins vary greatly and toxins may be cytotoxic, neurotoxic, hemolytic or cause necrosis. Of primary concerns are acute biological toxins.

Pertussis toxin is a protein-based exotoxin produced by the bacterium *Bordetella pertussis*, which is the causative agent of whooping cough. The LD₅₀ for humans is estimated to be 10 µg/kg or 700 µg if you weigh 155 lbs. Pertussis toxin has been shown to affect the innate immune response and is able to cross the blood-brain barrier, thereby causing neurological complications.

In the laboratory setting, typical routes of exposure are through inhalation, mucous membrane contact (eyes, nose and mouth) and/or to open sores on skin, sharps injuries with contaminated materials, and ingestion of trace amounts of the material if hands are not washed prior to eating or smoking.

A combined vaccine is available. It is thought that vaccination with pertussis vaccine is protective and will limit long-term side effects if parenteral exposure occurs. For workers in laboratories in which large amounts of toxin is typically used (over 100 µg), or in which toxin is routinely used with sharps, risks and benefits of vaccination should be discussed.

All personnel working with a biotoxin or accessing a toxin laboratory should be familiar with the signs and symptoms of toxin exposure.

Hazard statement(s)

- Causes skin irritation (H315)
- Causes serious eye irritation (H319)
- May cause respiratory irritation (H335)

Symptoms may include:

- Low blood sugar (shakiness, sweating, hunger, irritability)
- Difficulty breathing
- Impaired mental state (confusion, inability to complete routine tasks)

2. Laboratory Precautions

Standard Laboratory practices

Toxins shall be handled with appropriate precautions consisting primarily of **good microbiological laboratory techniques** as well as Biosafety Level 2 (BSL-2) containment. The following precautions should be employed:

- A. Access to the laboratory is limited or restricted at the discretion of the laboratory director.
- B. Placards should be placed on the entrances to the lab listing biological hazards and the PI's name and 24/7 contact information for the PI and/or laboratory personnel familiar with the biohazard.
- C. In addition, when performing work with toxins, access to the room should be restricted and a sign stating the following should be placed on the door: "Toxins in Use—Authorized Personnel Only."
- D. Do not store food in lab.
- E. Do not eat, drink, smoke, handle contact lenses, apply cosmetics (including chap stick), etc. in the lab.

- F. Do not mouth pipette.
- G. Plants or animals not involved in experiments are not allowed in the lab.
- H. Vacuum lines must be HEPA filtered
- I. Liquids should be handled carefully to minimize creation of splashes and aerosols. Centrifugation should be performed using sealed tubes and sealed rotors or safety cups.
- J. Sharps should be handled with extreme caution to avoid cuts or autoinoculation during use and disposal. Needles should not be bent, sheared, or recapped. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated by autoclaving.
- K. Transport: Toxin must be transported in a sealed primary container inside a sealed durable and leak proof secondary container that has been labeled with a warning label.
- L. Lab personnel must wash their hands after they handle the toxin and or animals injected with toxin, after removing gloves, and before leaving the laboratory or animal facility.
- M. Laboratory personnel must be appropriately trained.
- N. The safety protocol (SOP) serves as training documentation and reference information. A copy signed by laboratory personnel should be stored in the lab's safety manual.

Pertussis-specific laboratory and animal precautions

- A. Store in tightly closed container.
- B. Store locked up.
- C. All manipulations and dissections of animals injected with pertussis toxin must be performed within a Biosafety Cabinet (BSC).
- D. Animal studies: Following injection of pertussis toxin and for the duration of the experiment, animals must be placed in ventilated caging racks under negative pressure with HEPA-filtered exhaust. Alternate caging options may be determined in conjunction with DLAR and the BSO on a case-by-case basis with IBC approval.

2. Personal Protective Equipment (PPE)

- A. Wear protective gear including disposable gloves (double gloves recommended) and a dedicated cloth or disposable lab coat. Ensure gloves are compatible with any solvent your toxin may be dissolved in.
- B. Wear safety glasses and face protection when not working in a fume hood or biosafety cabinet.
- C. Dispose of contaminated PPE in biohazard bags/containers.
- D. No personal protective equipment shall be worn outside of the lab.

3. Working Procedures for Pertussis Toxin

Before working with toxin:

- Freshly prepare a solution of 10% bleach in water in appropriate containers for disinfecting supplies that may come in contact with the toxin.
- Put on PPE

While working with toxin:

- If at all possible, avoid working with lyophilized toxin (i.e. weighing of toxin, etc). Ideally, the toxin is purchased in solution. If you must weigh a toxin, then the scale must be located in a certified chemical fume hood.
- Sharps use:
 - Toxin may come in glass ampules that must be "snapped" to have access to the powder within for resuspension. Use great care in cracking the ampule to avoid an accidental cut.
 - Toxin may arrive in rubber septum sealed vials. If using a needle and syringe to plunge through the septum to resuspend, then use a hands-free device to stabilize the vial to avoid an accidental needle stick.
 - Whenever possible, use needle-free techniques to resuspend toxins
- Use a biological safety cabinet (BSC) for resuspension of biological toxins or manipulation of stock concentrations that can create aerosols, such as pipetting, harvesting or infecting cells, filling tubes and containers, and opening sealed centrifuge containers. Aerosol resistant tips must be used when pipetting.
- Immediately replace gloves, if contamination is suspected.

After working with toxin:

- **Toxins should NEVER be disposed of without prior inactivation.**
- Treat liquid waste with bleach to a final concentration of 10% bleach for a minimum contact time of 30 minutes. After 30 minutes, dispose of liquid waste in a sink with copious amounts of running water. Pertussis toxin is an environmental hazard. Do not allow inactivated toxin to enter the drain.
- Solid waste including pipettes, containers, etc. that come in contact with toxin must be disinfected with 10% bleach prior to disposal in biohazard waste container. Cavicide or ethanol are *not* effective disinfectants for inactivating pertussis toxin. Dispose of solid wastes in orange bags, which are autoclaved and placed in a red biohazard bag/container for final disposal.
- Following disposal of the liquid waste, rinse glassware with 10% bleach for 30 minutes, followed by washing and autoclaving.
- Disinfect work surfaces with 10% bleach. Bleach can corrode metal surfaces, but this can be avoided by wiping the surface with water or 70% ethanol after decontaminating to remove the bleach.
- Remove disposable PPE and dispose of it in biohazard bags.
- Wash hands with soap and water.

3. Emergency procedures

3A. Spills of the agent:

- 1) Notify workers in the area.
- 2) Leave the area for 15 minutes to allow aerosols to settle. Replace contaminated PPE.
- 3) Upon return, mix spill with freshly made bleach to 10% final concentration.
- 4) Allow 30 minutes of contact time for disinfection.
- 5) Absorb spill with paper towels and dispose them into biohazard bags.
- 6) Use dustpan and broom to sweep up debris. Broken glass must be deposited into broken glass or sharps box.
- 7) Wipe the spill area clean using 10% bleach.
- 8) Dispose of contaminated PPE in autoclavable biohazard bags.

3B. In the event of injury or exposure

- 1) **WOUNDS (including needle sticks and animal bites):** Wash all wounds immediately with antiseptic soap and a high volume of water for 5-15 minutes.
- 2) **SKIN:** Wash with plenty of soap and water for 5-15 minutes
- 3) **EYES:** Rinse cautiously with water for several minutes. Remove contact lenses if present. Continue rinsing for 15 minutes.
- 4) **INHALATION AND ACCIDENTAL INGESTION:** Rinse mouth but do not swallow. Do not induce vomiting. Fresh air and rescue breathing may be necessary as inhaled toxin can be fatal.
- 5) **SEEK IMMEDIATE MEDICAL FOLLOW-UP** (*do not wait 24 hrs*). Report exposures prior to any signs and symptoms developing, so that the use of hyperimmune globulin can be considered.

Employees *and* students go to:

- **Employee Health Services** (during business hours: Monday-Friday, 7:30 am -4 pm).
Address/Location: 57 Bee Street, Charleston SC 29425; Phone: (843) 792-2991
- **MUSC Emergency Room** (after business hours)
Address/Location: 96 Jonathan Lucas Street, Charleston SC 29425
Bring the MSDS and be prepared to discuss the nature of the toxin.

- 6) **REPORT EXPOSURE IMMEDIATELY** to the Principal Investigator and notify Biosafety Officer (843-792-3604).
- 7) **NOTIFY** Employee Health Services within 24 hours by filing a Workplace Injury form at [Care Team Member/Workplace Injury Form](#)

