 <b>UMDNJ</b> UNIVERSITY OF MEDICINE & DENTISTRY OF NEW JERSEY	<b>STANDARD OPERATING PROCEDURES</b>	<b>SOP #:</b> <b>REVISION:</b> <b>2005</b>
<b>TITLE: Use of Tetanus Toxin in the Laboratory</b>		<b>EFFECTIVE DATE:</b>
Employee Health Services Approval:	Date:	
EOHSS Approval:	Date:	
PI Review and Acceptance	Date:	

**Prior to working with tetanus toxin, all individuals will have a tetanus titer performed at the appropriate campus Occupational Medicine/Employee Health Services. A successful titer is indicated by greater than 0.10 IU/mL.**

**Tetanus toxin is a powerful neurotoxin which may be fatal if inhaled or introduced into a wound.<sup>7,3</sup> As a result, respiratory protection and skin protection are critical to safe handling of tetanus toxin.**

### 1. PURPOSE

- 1.1. This policy establishes procedures for safe handling, transport and storage of tetanus toxin (No CAS #). Tetanus toxin (also known as tetanospasmin) is an extremely potent neurotoxin. It has an LD50 in an unvaccinated person of < 2.5 nanograms/kg. This LD50 makes tetanus toxin among the most toxic materials known. It is critical that any individual involved in the handling of tetanus toxin has active immunization with adsorbed tetanus toxoid. This is normally regarded as administration at 10-year intervals.

### 2. SCOPE:

- 2.1. This SOP addresses the use of tetanus toxin in the laboratory and vivarium at UMDNJ. It assumes that all of the UMDNJ minimum safety requirements, as detailed in the latest version of the UMDNJ Laboratory Safety Plan have been implemented and that any individuals involved in the use or handling of tetanus toxin have up-to-date active immunization with adsorbed tetanus toxoid as indicated by a successful serum titer test. This SOP covers:
  - 2.1.1. Tetanus toxin.
  - 2.1.2. Any in vitro or in vivo system into which tetanus toxin is introduced. This includes in vitro work (tissue culture, molecular biology, tissue) and in vivo work (animals).

### 3. RESPONSIBILITY

- 3.1. All staff who will be engaged in the use or handling of tetanus toxin, or working in an area where tetanus toxin must first demonstrate a successful serum titer test. Additionally, these individuals are responsible for understanding all hazards associated with its use and for using appropriate personal protective equipment (PPE).

3.2. The Principal Investigator (\_\_\_\_\_) is responsible for ensuring that his/her staff has demonstrated successful serum titer tests and has been trained in the use, storage and handling of tetanus toxin.

3.3. Tetanus toxin use is restricted to work areas in building/room(s) \_\_\_\_\_ where staff members have up-to-date active immunization with adsorbed tetanus toxoid. Additionally, these staff members must have prior training in proper use, handling and storage of tetanus toxin. The individuals, who may work with tetanus toxin or be in the work area where tetanus toxin is used, handled or stored include:

_____	_____
_____	_____
_____	_____
_____	_____

3.4. Access is limited to personnel listed in 3.3 above, when experiments are in progress. A sign as described in \_\_\_\_\_ shall be posted on the door to inform Environmental Services, Physical Plant and all other personnel not involved in the protocol to not enter the room.

#### 4. PROCEDURES

##### 4.1. Training

- 4.1.1. Prior to conducting any work with tetanus toxin, the Principal Investigator or his/her designee must provide training to laboratory personnel regarding the hazards involved in working with this substance, work area decontamination, and emergency procedures.
- 4.1.2. The Principal Investigator must provide his/her laboratory personnel with a copy of this SOP and a copy of the tetanus toxin MSDS (see attachment to this SOP).
- 4.1.3. The Principal Investigator must ensure that his/her laboratory personnel each have a successful titer to the tetanus toxin. A successful titer is indicated by greater than 0.10 IU/mL of serum using the enzyme immunoassays (EIA).
- 4.1.4. The Principal Investigator must ensure that his/her laboratory personnel have attended initial laboratory safety training or refresher training within the last two years.
- 4.1.5. The Principal Investigator must ensure that his/her laboratory personnel have been evaluated to insure that they can be safely administered the tetanus immune globulin (TIG) and tetanus toxoid if an exposure to tetanus toxin is reported or believed to have occurred. Accidental parenteral inoculation and ingestion of the toxin is known to be a concern. For more information on the administration of

TIG and tetanus toxoid, see the following websites:

<http://www.univgraph.com/bayer/inserts/baytet.pdf>

<http://www.vaccineinformation.org/tetanus/recommen.asp>

**Note:** EOHSS is available to assist in the education and/or training of personnel concerning safe work practices.

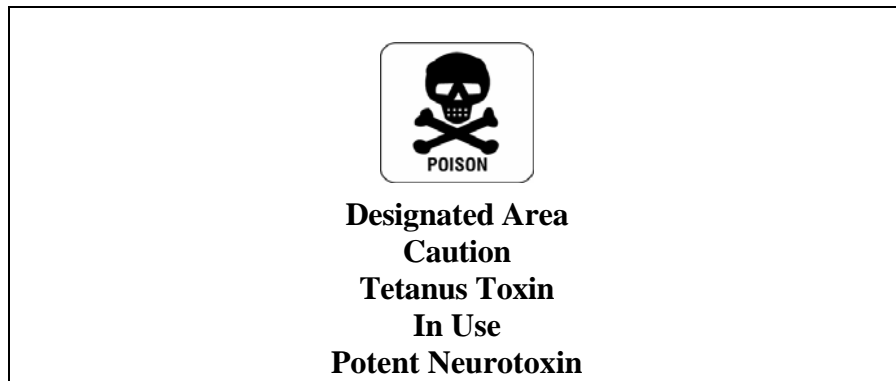
#### 4.2. Signage

4.2.1. When tetanus toxin is in use, warning signs must be posted on the laboratory door, chemical hood, and the biological safety cabinet (BSC) until the tetanus toxin has been returned to storage and the work area has been decontaminated.

4.2.2. A sign must be posted on animal room door/s and on animal cages that contain animals administered tetanus toxin.

4.2.3. Signs will include the information shown in the following samples:

#### **Sample Cage, Chemical Hood, BSC Sign**



#### **Sample Door Sign For When Tetanus Toxin Is In Use**



## **STOP: DO NOT ENTER**

**Tetanus Toxin In Use—Potent Neurotoxin  
Authorized Personnel Only**  
**Material Safety Data Sheet is available in Room \_\_\_\_\_**  
**For More information, please contact: \_\_\_\_\_**  
**In the event of a spill, contact Public Safety at \_\_\_\_\_**  
**and ask for EOHSS to respond.**

### 4.3 Storage

- 4.3.1 Lyophilized tetanus toxin powder or solutions containing tetanus toxin must be stored in a locked storage room, cabinet, refrigerator or freezer when not in use.
- 4.3.2 Store tetanus toxin in appropriate, sealed containers with unbreakable secondary containment (i.e., a bottle or vial within a sealed plastic jar). Label all containers, including secondary containment, with “tetanus toxin” and the warning “Danger-potent neurotoxin.” **Do not freeze tetanus toxin powder.** This product is stable for years in the dried form, when stored at 4-7 degrees C.
- 4.3.3 An inventory of all tetanus toxin must be maintained, noting amounts ordered, stock on hand and amounts used. [Note: Tetanus toxin is not subject to the select agent registry program, (42 CFR Part 73).]

### 4.4 Handling and Solution Preparation

- 4.4.1 When handling the tetanus toxin in the lyophilized form (powder), wear an N-100 filtering facepiece. In order to weigh out lyophilized tetanus toxin for dissolution tare a closed vessel on a balance outside the chemical hood. Add the tetanus toxin in the chemical hood, and reweigh the closed vessel outside the hood. Prepare one stock solution of tetanus toxin, adequate for all subsequent anticipated procedures. The handling of the stock solution and any subsequent dilutions must take place in the chemical hood. Prepare the smallest amount of tetanus toxin solution necessary for the specific procedure.

- 4.4.2 Biosafety level 2 (BSL-2) practices will be used for all activities involving tetanus toxin (including the introduction of tetanus toxin into in vitro systems, e.g., cell cultures, and for in vivo work, e.g., mice, rats), unless the toxin-containing materials have been inactivated. Please fill out and attach the UMDNJ BSL-2 safety checklist to EOHSS

(<http://www2.umdj.edu/eohssweb/publications/BL2.htm>) If use of a needle is necessary, a safety device/blunt tipped needle shall be used to decrease the possibility of inadvertent self inoculation with tetanus toxin. The work will be performed in a Class II biological safety cabinet in a manner consistent with biosafety level 2 work (BSL-2) as provided in Sections III. of the CDC/NIH publication, Biosafety in Biomedical and Microbiological Laboratories, 4<sup>th</sup> edition. BSCs will meet or exceed performance criteria as provided in NSF Standard 49. Onsite field verification of any BSCs used for the handling of tetanus toxin will be performed at least annually in a manner consistent with NSF Standard 49.

For additional information on biological safety cabinets see:

<http://www2.umdj.edu/eohssweb/aiha/technical/biosafety.htm#Cabinets>

For additional information on blunt tip safety needles see:

<http://www.infectioncontrolday.com/articles/321feat3.html>

<http://www.abbotthosp.com/NPS/contact.htm> (Actual sites for blunt tip safety needle products from Abbott Labs are under construction.)

<http://catalog.bd.com/bdCat/viewProduct.doCustomer?productNumber=305211>

- 4.4.3 Work with tetanus toxin will take place over spill trays or disposable plastic-backed absorbent paper whenever possible. Spill trays will be decontaminated using the procedures in Section 4.4.12. Absorbent paper will be decontaminated using the procedures in Section 4.4.13.
- 4.4.4 Use disposable lab ware whenever possible. Disposable lab ware should be decontaminated with a 0.1N sodium hydroxide (NaOH) solution followed by decontamination with a 1:2 dilution of 5.25% sodium hypochlorite bleach before discarding in a red biohazard bag and placed in a gray regulated medical waste (RMW) container. Reusable lab ware must be decontaminated with a 0.1 N NaOH solution followed by decontamination with a 1:2 dilution of 5.25% sodium hypochlorite bleach, rinsed with water, and autoclaved at 121 degrees C and 15 psi for 15 minutes.
- 4.4.5 Use of sealed rotors or safety cups is required when solutions containing tetanus toxin are centrifuged (unless the toxin has been inactivated).

- 4.4.6 When expelling air from syringes used for administering tetanus toxin solutions, wrap the tip of the needle with sterile dampened gauze. After injection, do not clip, remove, or recap the needle. Instead, place used syringes directly into a container with a 1:2 dilution of 5.25% sodium hypochlorite bleach, let it remain for 30-minutes. Transfer to a sharps container that has been previously placed in the BSC. Check the injection site for leaking material and absorb with dampened gauze. Contaminated gauze must be placed in a container with a 1:2 dilution of 5.25% sodium hypochlorite bleach, let it remain for 30-minutes. Transfer to a red biohazard bag and autoclave waste materials at 121 degrees C and 15 psi for 15 minutes. Place in a gray RMW container for disposal.
- 4.4.7 Two individuals trained per Section 4.1 must be present whenever a high risk operation will be conducted. High risk operations include handling of tetanus toxin in a syringe with a needle. Each individual must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.
- 4.4.8 Tetanus toxin containers to be removed from the chemical hood or BSC must be decontaminated (see Section 4.4.11) and placed in a labeled, unbreakable secondary container (see Section 4.3.2) prior to removal. The secondary container will be decontaminated prior to removal from the laboratory.
- 4.4.9 When vacuum lines are used with systems containing tetanus toxin, they must be protected with a HEPA filter (Whatman Vacu-Guard Filter No.:6722-5000) to prevent entry of toxin into the lines. Sink drains must be protected in a similar fashion when water aspirators are used.
- 4.4.10 The following minimum personal protective equipment must be worn during operations with tetanus toxin powder and its solutions:
- 4.4.10.1 Safety glasses with side shields or safety goggles.
  - 4.4.10.2 Disposable impermeable gloves. Double-gloving is recommended (latex, latex/nitrile or nitrile). Change gloves frequently and when contaminated, punctured or torn. Wash hands immediately after removing gloves.
  - 4.4.10.3 The use of a NIOSH-approved N-100 disposable filtering facepiece respirator is required when working with tetanus toxin powder or tetanus toxin in solution if there is a concern for any aerosol. Contact EOHSS, <http://www2.umdnj.edu/eohssweb/eohss.htm#Contact>, for guidance on respirator ordering, use and selection.
  - 4.4.10.4 A disposable laboratory coat, jumpsuit or other appropriate protective clothing will be worn during work with tetanus toxin. The disposable laboratory coat will be placed in a red biohazard bag by an individual outfitted in a NIOSH approved N-100 filtering facepiece respirator and autoclaved at 121 degrees C and 15 psi for 15 minutes. The red biohazard bag along with will go out as regulated medical waste.

- 4.4.11 Use of a 0.1N NaOH solution followed by a 1:2 dilution of 5.25% sodium hypochlorite bleach is recommended for decontamination of tetanus toxin. A 30-minute exposure time is recommended for each solution. Decontamination solutions should be prepared on a weekly basis at a minimum to ensure adequate concentration.
- 4.4.12 The chemical hood and BSC must be disinfected with a 0.1N NaOH solution followed by a 1:2 dilution of 5.25% sodium hypochlorite bleach after the use of tetanus toxin. Following the wiping of the internal hood surface, spray and wipe with a 70% ethyl alcohol or 70% isopropyl alcohol solution. (Note: Repeated wiping of stainless steel surfaces with bleach solution can result in pitting of the stainless steel.) Additionally all equipment must also be disinfected with a 0.1N NaOH solution followed by a 1:2 dilution of 5.25% sodium hypochlorite bleach after the use of tetanus toxin.
- 4.4.13 Remaining solutions of tetanus toxin will be inactivated by autoclaving. In the chemical hood, loosen the cap of the primary container to allow steam penetration. Place the primary container into a secondary biohazard sharps container and place the sharps container in an autoclavable pan. Autoclave at 121 degrees C and 15 psi for 60 minutes on liquid cycle (slow exhaust). Discard the primary container and sharps container as RMW.
- 4.4.14 Dry waste (paper towels, plastic-backed absorbent paper, gloves) must be decontaminated with a 0.1 N NaOH solution followed by a 1:2 dilution of 5.25% sodium hypochlorite bleach. The materials will then be disposed of as RMW.
- 4.4.15 Wash hands thoroughly immediately after working with any concentration of tetanus toxin.

## 5 Animal Handling and Housing

### 5.1 General Procedures

- 5.1.1 Approval from the school/unit IACUC is required before use in animals.
- 5.1.2 Animal cages containing animals treated with tetanus toxin must be labeled in accordance with the sample label shown in 4.2.3.
- 5.1.3 Mice or rats treated with tetanus toxin must be handled using BSL-2 practices. Work with mice or rats will be performed in a Class II BSC. Mice or rats will be housed and handled within static micro-isolator cages or HEPA-filtered vented cage racks or equivalent.
- 5.1.4 Use low-dust bedding such as corncob or paper-type.
- 5.1.5 All potentially contaminated surfaces must be decontaminated with a 0.1N NaOH solution followed by a 1:2 dilution with 5.25% sodium hypochlorite bleach. Paper towels or rags used for cleaning must be decontaminated per Section 4.4.13 and placed in a RMW container.

## 5.2 Cage Changing

- 5.2.1 Cage changing will be performed in a Class II BSC only by appropriately trained staff.
- 5.2.2 Wear the personal protective equipment specified in 4.4.5, including a NIOSH-approved N-100 respirator.
- 5.2.3 Prior to cage cleaning and dumping of bedding, whole cage and contents should be placed into autoclave bag, autoclaved at 121 degrees C and 15 psi for 60 minutes. Place in a gray RMW container for disposal.

## 6 EMERGENCIES

### 6.1 Spill of tetanus toxin outside of the chemical hood or BSC:

- 6.1.1 Phone the Public Safety Emergency number \_\_\_\_\_ immediately. Ask them to have EOHSS respond. **DO NOT** attempt to clean up a spill.
- 6.1.2 Isolate the area to prevent the spread of contamination, e.g., close doors to affected area and post a warning sign.
- 6.1.3 Alert personnel in the immediate area to evacuate.
- 6.1.4 Attend to any injured personnel.

**NOTE:** Personnel must not attempt to clean up a spill of tetanus toxin powder or tetanus toxin solution which occurs outside of a chemical hood or BSC.

### 6.2 Spill of tetanus toxin inside the chemical hood or BSC:

- 6.2.1 Small spills may be decontaminated by gently covering the spill with paper towels and flooding the area (starting at perimeter and working towards the center) to arrive at a solution of 0.1N NaOH. Allow sufficient contact time (30-minutes) before clean up. Next, clean the spill area with a 1:2 dilution of 5.25% sodium hypochlorite bleach, allowing a 30-minute contact time. Place waste materials in a red biohazard bag and autoclave waste materials at 121 degrees C and 15 psi for 15 minutes. Place in a gray RMW container for disposal.
- 6.2.2 Notify EOHSS of the spill and the cleanup procedure.
- 6.2.3 If the spill is large or you are unsure of your ability to thoroughly decontaminate it, call the Public Safety Emergency number (above) and ask EOHSS to respond.

### 6.3 Accidental Exposure to Tetanus Toxin

- 6.3.1 In the event of a recognized percutaneous or mucous membrane exposure to tetanus toxin:
  - 6.3.1.1 Tetanus immune globulin (TIG) must be taken as soon as possible following percutaneous or mucous membrane exposure. Notify the

campus Occupational Medical/Employee Health Services at \_\_\_\_\_ or go to the nearest emergency room. If the Tetanus immune globulin is not available, tetanus antitoxin (equine origin) following testing for hypersensitivity must be taken. Mechanical assistance with breathing may be necessary.

6.3.1.2 Remove contaminated clothing.

6.3.1.3 Rinse the affected area with water, using a safety shower or eyewash, as appropriate, for a minimum of 15-minutes. A disposable laboratory coat or jumpsuit should be available for the victim to wear after using a safety shower.

6.3.1.4 Notify the victim's supervisor, if immediately available. The supervisor, a co-worker, or the victim must contact Employee Health at \_\_\_\_\_ to determine what additional steps should be taken.

6.3.1.5 If the incident occurs off-hours, or an ambulance is needed because of injury, contact Public Safety at (phone number) \_\_\_\_\_ to advise them of the medical emergency.

6.4 In the event of a suspected or incidental exposure to tetanus toxin:

6.4.1 Immediately rinse the affected area using a safety shower or eyewash for a minimum of 15-minutes.

6.4.2 Notify the supervisor if immediately available. The supervisor, a co-worker, or the victim must contact Employee Health at \_\_\_\_\_ to determine what additional steps should be taken.

6.4.3 If the incident occurs off-hours, or an ambulance is needed because of injury, contact Public Safety at (phone number) \_\_\_\_\_ to advise them of the medical emergency.

## 7 REFERENCES

7.1 Shimoni Z, Dobrousin A, Cohen J et al. Tetanus in an immunised patient. BMJ 1999; 319:1049.

7.2 CDC/NIH Biosafety in Biomedical and Microbiological Laboratories, 4<sup>th</sup> edition,

7.3 List Biological Laboratories MSDS for tetanus toxin product #190, [www.listlabs.com/msds/190.htm](http://www.listlabs.com/msds/190.htm)

7.4 Baytet, Tetanus Immune Globulin (Human), Bayer Corporation, revised October 2000.

7.5 Gill, D. Michael; 1982; Bacterial toxins: a table of lethal amounts; Microbiological Reviews; 46: 86-94.

7.6 Health Canada MSDS for Clostridium tetani, updated January 23, 2001, <http://www.phac-aspc.gc.ca/msds-ftss/msds38e.html>