

Standard Operating Procedures: Staphylococcus Enterotoxins

Staphylococcal enterotoxin (SE) strains are categorized by the CDC and USDA as ‘Select Agent’ toxins, requiring registration with the CDC at concentrations greater than 5 mg. Concentrations below 5 mg can be used and stored in a laboratory at Rutgers without CDC registration but prior to acquisition and use, the use of SE must be registered with the RU Institutional Biosafety Committee (IBC). Additionally, the PI must verify that the quantity required for research is below the restricted threshold. It is recommended that an inventory be maintained to document that the laboratory never exceeds the permitted amount of toxin. If you have any questions or need guidance please contact the Campus Biosafety Officer at Biosafety@rutgers.edu.

Principal Investigator (print):	
Principal Investigator Signature:	
Date Reviewed:	
Location:	Rutgers University
Campus:	
Building:	
Designated Use Area / Room(s):	
Designated Storage Area/Room	
IBC Approval Number:	
IACUC Approval Number (If applicable)	

Physical Characteristics:

The Staphylococcal enterotoxins (SE) represent a group of proteins which are secreted by Staphylococcus aureus. They play a role in the pathogenesis of food poisoning and are the most potent known activators of T lymphocytes. At present, seven enterotoxins are known, namely A, B, C1, C2, C3, D and E. Their molecular masses range between 27 and 30 kDa. Staphylococcus enterotoxin type A or ‘SEA’, is the most common toxin implicated in staphylococcal food poisoning.

In this laboratory, the following strain(s) of SE is used:

Health Hazard Summary:

Illness is characterized by high fever, hypotension, diarrhea, shock, and in some cases death.

Safety Data Sheet (SDS): (Attach manufacturer-specific SDS to this SOP)
Read the manufacturer's SDS, formerly called the material safety data sheet (MSDS), and maintain a copy in your safety binder along with this SOP. For safety questions, contact Rutgers Environmental Health & Safety (REHS) at 848-445-2550.

Personnel Requirements:

Immunocompromised personnel should avoid work with SE. All laboratory members must be aware of the signs and symptoms of SE infection.

Exposure Control:

- Purchase SE in small quantities.
- Handle SE in a certified biosafety cabinet (consult with REHS).
- Wear double nitrile gloves, eye protection and lab coat when handling SE.
- Use syringe with integral safety feature, as applicable.
- Keep a solution of 10% bleach solution readily accessible (made fresh daily).
- Anesthetize/restrain animals, as applicable.
- Avoid inhalation and physical contact with SE.
- Ensure that a safety shower and eyewash station are nearby.

First Aid Procedures:

- Call for medical advice immediately:
 - Occupational Medicine Services (Newark) 973-972-2900
 - Hurtado Health Center (New Brunswick) 848-932-8254
 - Emergencies & After Hours – Call the Rutgers University Police Department (RUPD) or visit nearest hospital Emergency Room
 - 732-932-7211 (Piscataway & New Brunswick)
 - 973-972-4490 (RBHS - Newark / Scotch Plains)
 - 973-353-5111 (Rutgers-Newark)
- Additional first aid based on route of exposure:
 - Ingestion/oral exposures – rinse mouth with water.
 - Inhalation exposure – move person to fresh air and call for an ambulance if breathing becomes difficult.
 - Contact exposure (eyes, nose, skin) – flush the affected area with copious amounts of water for at least 15 minutes.
 - Accidental Injection / Percutaneous – call RUPD and request an ambulance or go to the nearest hospital emergency room.

Injury / Exposure Reporting:

Any exposure incidents must be reported in the REHS Accident Database located online at <http://myrehs.rutgers.edu>. The injured/exposed person's direct supervisor (e.g., PI or lab manager) needs to submit the incident report by the end of the work shift.

Spill Clean-up:

For small quantities (less than 5ml or 100 ug).

- If you don't feel comfortable cleaning up the spill, follow the instructions for large spills (below).
- Wear double nitrile gloves, lab coat, and safety glasses/goggles.
- Any broken glass fragments should be picked up with tongs, forceps or a small scoop (never use your fingers). Place the broken glass in a wide-mouthed plastic container. Tightly seal the container and contact REHS (<http://rehs.rutgers.edu>) for disposal.
- Liquids should be absorbed with paper towels and saturated with 10% bleach solution – 60 minute contact time!
- Solids should be wiped up with wetted paper towels saturated with a 10% bleach solution – 60 minute contact time!. Contaminated surfaces should then be cleaned three times using a detergent solution and paper towels followed by clean water.
- Inside a ducted hood, contaminated re-usable items (e.g., glassware and scoops) should be disinfected with a 10% bleach solution, washed three times with detergent by a trained employee wearing two pairs of nitrile gloves, eye protection and fully fastened lab coat or gown.
- Contaminated disposable items & spill clean-up waste (gloves, paper towels, absorbent pads, spill pads/pillows) must be bagged and autoclaved at 121°C and 15 psi for 60 minutes on liquid cycle (slow exhaust). The materials must then be disposed as biomedical waste.
- If your building does not have an autoclave, collect all spill clean-up materials in tightly sealed containers, and contact REHS (<http://rehs.rutgers.edu>) for disposal.

For large spills (greater than 5ml or 100 ug) or possible airborne SE:

- Evacuate the area.
- Report the spill to Rutgers University Police Department (RUPD)
 - 732-932-7211 (Piscataway & New Brunswick)
 - 973-972-4490 (RBHS - Newark / Scotch Plains)
 - 973-353-5111 (Rutgers-Newark)
- The police dispatcher will contact on-call REHS personnel.
- REHS staff will clean-up the spill.

General Safety Precautions:

1. Handle SE liquid suspensions/cultures in a certified biosafety cabinet, wearing personal protective equipment (PPE): lab coat, safety glasses, and nitrile gloves.
2. Place absorbent pad in the bottom of the cabinet to contain potential spills.
3. Aliquot 5ml of a 10% bleach solution into a conical tube. Place the open tube in a tube rack to serve as waste receptacle for contaminated filtered pipette tips – minimum of 30 minute contact time!
4. Use extreme caution when preparing/handling needles of SE. Use needles with integral safety feature (e.g., BD Safety Glide™). Dispose of contaminated needles immediately in sharps container.
5. Animal Administration: restrain or anesthetize animal during the injection, label the cage card with a biohazard label and SE information, maintain cages in unventilated microisolator cage racks in Vivarium and in the ducted chemical hood inside the laboratory, wear PPE when handling the animals, and collect carcasses of SE-

- administered animals in a separate bag with a biohazard warning label and SE information when returning carcasses to the research animal facility for disposal.
6. Animal Housing: use static or microisolator cages. Never use ventilated cage racks without first consulting with REHS.
 7. Cages and Bedding: First bedding change (minimum 72-hour post-dosing):
 - Performed by laboratory personnel in biosafety cabinet.
 - Autoclave cages and bedding
 - Use biosafety cabinet to empty autoclaved bedding.
 - Dispose autoclaved bedding in biomedical waste container after first administration.
 - Subsequent changes performed by CMR staff. No special handling precautions.
 8. Carcass Disposal: place in red biohazard bag and then into Vivarium biohazard freezer for incineration.
 9. Inactivate DT stocks and DT-contaminated items by autoclaving (121°C and 15 psi for 60 minutes) or chemical inactivation with sodium hypochlorite / bleach (30 minutes of contact time with liquid bleach) prior to disposal. Surfaces may be decontaminated with bleach.

Lab-Specific Procedures & Safety Precautions (to be completed by Principal Investigator). You may attach separate pages if more space is required:

Materials: List manufacturer, catalog number, quantity to be ordered and form of material – e.g., lyophilized powder.

Preparation: List specific steps for preparing aliquots, specify containment controls, PPE worn, disinfection steps for equipment used, storage information.

Procedure for Use in Mice (if applicable): Include description of containment controls, injection dilution, method of injection, dosing and cage marking information, carcass disposal, etc.

Procedure for Use In-Vitro (if applicable): Specify containment controls used, describe preparation of cell culture and how cell culture is treated and disposed.

Signatures:

By signing below, I certify that I have read this SOP and attached material, that I understand the procedures for working with diphtheria toxin, that I understand the hazards associated with using diphtheria toxin, and that I will use the procedures described in this SOP to safely handle and use diphtheria toxin.

Name (typed)	Job Title	Signature