

**Standard Operating Procedures: Clostridium difficile**

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 *Clostridia* species are gram-positive, rod-shaped, spore-formers. They are generally obligate anaerobes and are ubiquitous saprophytes or part of the normal flora. *Clostridium difficile* is an opportunistic pathogen that produces large, oval, subterminal spores and two toxins. Toxin A is an enterotoxin that causes fluid accumulation in the intestine while toxin B is a cytopathic agent. *C. difficile* flourishes when antibiotics eliminate the normal intestinal flora.

**Health Hazard Summary:**

Opportunistic pathogen, broad-spectrum antibiotic therapy eliminates competing gut flora, allowing the overgrowth of *C. difficile*; important cause of antibiotic-associated diarrhea and pseudomembranous colitis; diarrhea in cancer patients receiving chemotherapy; symptoms range from mild diarrhea to severe colitis (possibly fatal).

**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 *C. difficile*. All laboratory members must be aware of the signs and symptoms of *C. difficile* infection.

### **Exposure Control:**

- Purchase *C. difficile* in small quantities.
- Handle *C. difficile* in a certified biosafety cabinet (consult with REHS).
- Wear double nitrile gloves, eye protection and lab coat when handling *C. difficile*.
- 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 *C. difficile*.
- 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 – 20 minute contact time!
- Solids should be wiped up with wetted paper towels saturated with a 10% bleach

- solution – 20 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 *C. difficile*:

- 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 *C. difficile* 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 *C. difficile*. 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 *C. difficile* 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 *C. difficile*-administered animals in a separate bag with a biohazard warning label and *C. difficile* 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 LAS 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.

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

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**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.

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

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**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