UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
ENVIRONMENT, HEALTH, AND SAFETY/BIOSAFETY

DIPHTHERIA TOXIN EXPOSURE/INJURY RESPONSE PROTOCOL

Organism or Agent: Diphtheria Toxin (C. diphtheriae)
Exposure Risk: Diphtheria/Myocarditis & Polyneuritis
Exposure Hotline: 415/353-7842 (353-STIC) (Available 24 hours)
Office of Environment, Health & Safety: 415/476-1300 (Available during work hours)
415/476-1414 or 9-911 (Emergency, 24 hours)
EH&S Public Health Officer: 415/514-3531
UCSF Occupational Health Services: 415/885-2824
Campus Biosafety Officer: 415/514-2824
California Poison Control: 800/222-1222
SFDPH Emergency Number: 415/554-2830
CDC Emergency Operations: 770/488-7100

PROTOCOL SUMMARY

In the event of an accidental exposure or injury, the protocol is as follows:

1. Modes of Exposure:
   a. Skin puncture or injection
   b. Ingestion
   c. Contact with mucous membranes (eyes, nose, mouth)
   d. Contact with non-intact skin
   e. Exposure to aerosols
   f. Respiratory exposure from inhalation of toxin

2. First Aid:
   a. **Skin Exposure**, immediately go to the sink and thoroughly wash the skin with soap and water. If working with diphtheria, decontaminate any exposed skin surfaces with an antiseptic scrub solution.
   b. **Skin Wound**, immediately go to the sink and thoroughly wash the wound with soap and water and pat dry.
   c. **Splash to Eye(s), Nose or Mouth**, immediately flush the area with running water for at least 5-10 minutes.
   d. **Splash Affecting Garments**, remove garments that may have become soiled or contaminated and dispose as chemical waste.

3. Treatment:
   a. In the event of an acute injury or exposure resulting from a laboratory incident, the injured employee/student should report to the Emergency Department (ED) for medical treatment. The injured individual must inform their supervisor, and take a copy of this entire protocol document to the ED, including information regarding the specific toxin subtypes associated with exposure.
   b. In the event of exposure, with or without an injury requiring immediate medical care, call the Exposure Hotline in order to get access to medical care specific to the exposure. The hotline...
responder will provide guidance to the injured individual on necessary medical treatment and post exposure follow-up.

4. **Follow-up is needed in the event of any Laboratory Exposure:**
   a. In the event of a large spill in a secure area, leave the area and secure the lab to prevent entry of other personnel, and possible secondary exposures. In the event of a spill in a non-secure area, contact the emergency response team (9-911) for clean-up.
ROLES & RESPONSIBILITIES
AFTER ACCIDENTAL EXPOSURE TO DIPHTHERIA TOXIN

1. WORKER’S RESPONSIBILITIES (Employee/Student Initial Self-Care)
   a. First Aid: Perform recommended first aid and decontamination according to the posted instructions. Decontaminate any exposed skin surfaces.
   b. Treatment: i) In the event of an acute exposure or injury resulting from a laboratory accident, the injured individual should report to the Emergency Department for acute medical treatment. The employee should bring a copy of this protocol. The employee should inform the ED physician of the exact types of toxin to which he/she was exposed. ii) In the event of an exposure, with or without an injury, call the Exposure Hotline in order to get access to medical care for the exposure and evaluation for possible post exposure prophylaxis.
   c. Access to the Exposure Hotline: Immediately, call the Exposure Hotline in the event of an exposure. Dial 415/353-7842 and provide your name and contact information to the operator. If there is no call back in 15 minutes, call again. If there is no call back the second time, proceed to the nearest Emergency Department with a copy of this protocol.
   d. Reporting: Inform your laboratory supervisor/principal investigator of the exposure.
   e. Secure the laboratory: Identify the equipment involved in the exposure and the mechanism of exposure. Make sure that the laboratory area has been secured and that notification of contamination has been posted to prevent other individuals from entering the area.
   f. Remove any garments that may have become soiled/contaminated, and dispose as chemical waste.
   g. Follow up: Contact Occupational Health Services (OHS) at 415/885-7580 for any needed follow-up care.

2. SUPERVISOR’S RESPONSIBILITIES
   a. First Aid and Decontamination: Verify that the worker has washed and decontaminated himself/herself. Ensure that appropriate medical treatment has been received.
   b. Secure the laboratory: Confirm that the laboratory area has been secured and that notification of contamination has been posted to prevent other individuals from entering the area.
   c. Laboratory clean-up (as needed): Contact the Office of Environment, Health & Safety (OEH&S) through the UC Police Department Emergency Dispatch (from a campus telephone 9-911, from a non-campus phone 415/476-1414).
   d. Report the exposure: Call the Public Health Officer during regular business hours to discuss the exposure. A report summarizing any suspected diphtheria toxin exposure needs to be submitted to the Biosafety Committee by the Principal Investigator (PI). The report must include the following:
      • A brief description of the exposure event, a description of the area involved, and the extent of employee exposure
      • If applicable, specification of the amount of toxic material released, time involved, and explanation of procedures used to determine the amount involved
      • Corrective action taken to prevent the re-occurrence of the incident
      • Decontamination procedures
The exposure incident should also be reported to the San Francisco Department of Public Health as required by the Cal/OSHA ATD standard.

e. **Follow-Up:** Confirm that the worker has called for an appointment at the UCSF Occupational Health Clinic.


3. **PRINCIPAL INVESTIGATOR RESPONSIBILITIES**
   a. The PI will ensure that all lab personnel are trained in the use of safe laboratory procedures to prevent accidental exposure before assignment to any laboratory where diphtheria toxin is used.

   b. The PI will carefully explain the necessity of immunization with diphtheria toxoid. The PI will ensure that all laboratory workers are either fully immunized against diphtheria, or sign a written declination form.

   c. The PI may request assistance from UCSF OEH&S in providing information about safe laboratory procedures and the necessity of immunization with diphtheria toxoid. For assistance, the PI should call the UCSF Public Health Officer or Biosafety Officer.

   d. The PI must ensure that all researchers who will be working with diphtheria toxin have read the entire protocol. The PI will also ensure that the protocol will be reviewed on a yearly basis by all laboratory workers.

   e. The PI shall be aware of the provisions of the California Aerosol Transmissible Disease Standard, since C. diphtheriae is a covered entity. For information, please refer to the California Code of Regulations: [http://www.dir.ca.gov/Title8/5199.html](http://www.dir.ca.gov/Title8/5199.html). The PI shall ensure that any known exposure is reported to the Biosafety officer and to the San Francisco Department of Public Health.

4. **EMERGENCY DEPARTMENT RESPONSIBILITIES**
   a. There have been reports of rapid onset local pain after percutaneous exposure to toxin, and such an occurrence would indicate a significant exposure. Onset of symptoms following significant toxin exposure would typically have onset delayed by days to weeks, and are due to the inhibition of protein synthesis. The Emergency Department shall assess the severity of the exposure, and take appropriate actions to include consultation with the California Poison Control System. Treatment with immune globulin may be considered in the absence of symptoms in case of an especially severe or large exposure.

   b. Diphtheria immune globulin is currently available from the CDC. In the event of problems obtaining a response at any local or state level, the CDC Emergency Operations Center should be contacted at (770) 488-7100. Contraindications/Precautions in the use of equine immune globulin include a history of prior exposure to horse serum, prior history of serum sickness, or a history of asthma or hay fever, especially when near horses.

   c. The emergency room should draw at least ten milliliters of serum and hold it for possible toxin assay. This must be done before any treatment with antitoxin.

   d. Any patient seen in the Emergency Department and released should be given information about the potential for delayed onset of symptoms/toxicity. Any symptoms would be reason for emergent reevaluation. Any exposed individuals should also be referred to the UCSF Occupational Health Services for follow-up care.
The Emergency Department and UCSF Occupational Health Services both need to complete a Doctor’s First Report of Occupational Illness (DFR). The Emergency Department physician should leave a message for Occupational Health that an exposure has occurred. The physician administering care should forward a copy of the DFR to UCSF HR. Here is a link to the form: [http://www.dir.ca.gov/dlsr/dlsrform5021.pdf](http://www.dir.ca.gov/dlsr/dlsrform5021.pdf)
MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: *Corynebacterium diphtheriae*

SYNONYM OR CROSS REFERENCE: Diphtheria

CHARACTERISTICS: Gram positive rod, non-sporulating, non-motile, characteristic swelling at one end of bacillus (club shaped), facultative anaerobe, metachromic granules, three biotypes - gravis, mitis, intermedius; produce toxin

SECTION II - HEALTH HAZARD

PATHOGENICITY: Two types of clinical infection: nasopharyngeal and cutaneous. Main manifestation is an upper respiratory tract infection, characterized by pharyngitis, fever, malaise, swelling of the neck and headache. Hypoxia may develop due to airway obstruction by the pseudomembrane; characteristic lesion marked by patch of grayish membrane with surrounding inflammation is hallmark of cutaneous infection; many inapparent infections; late effects of toxin after 2-6 weeks include cranial, motor and sensory nerve palsies and myocarditis, endocarditis; case fatality rate of 5-10% for noncutaneous diphtheria.

EPIDEMIOLOGY: Normal flora of skin and nasopharynx; disease of colder months in temperate zones, involving unimmunized children; found in adults whose immunization was neglected; in the tropics, seasonal trends are less distinct; inapparent, cutaneous and wound diphtheria cases are much more common.

HOST RANGE: Humans

INFECTION DOSE: Toxin is extremely potent

MODE OF TRANSMISSION: Direct respiratory or physical contact with patient or carrier; more rarely with articles soiled with discharges from lesions of infected persons; raw milk has served as a vehicle

INCUBATION PERIOD: Usually 2-5 days, occasionally longer

COMMUNICABILITY: Variable period, until virulent bacilli have disappeared from discharges and lesions (2 weeks or less); rare chronic carriers may shed organisms for 6 months or more; appropriate antibiotic therapy terminates shedding promptly
SECTION III - DISSEMINATION

RESERVOIR: Humans

ZOOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Sensitive to erythromycin and penicillin

DRUG RESISTANCE: Erythromycin-resistant isolates have been reported

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite and 70% ethanol, glutaraldehyde, formaldehyde, iodines

PHYSICAL INACTIVATION: Inactivated by moist heat (121°C for at least 15 min) and dry heat (160-170°C for at least 1 hour)

SURVIVAL OUTSIDE HOST: Air - 2.5 hours; carcass - 14 days; dust -7-102 days; exudate (infectious croup membrane) - up to 150 days; contaminated wooden toy - 180 days; soil - 1 year;

SECTION V - MEDICAL

SURVEILLANCE: Surveillance for lesions and formation of pseudomembrane; confirmation by culturing

FIRST AID/TREATMENT: If diphtheria is strongly suspected, antitoxin should be given without awaiting bacterial confirmation; antibiotic therapy should be administered after cultures in conjunction with antitoxin.

IMMUNIZATION: Immunization in young children with diphtheria toxoid (usually combined with tetanus toxoid and pertussis vaccine - DTP); primary immunization in adults with TD; protection maintained in adults and children by administering a dose of Tdap every 10 years.

PROPHYLAXIS: Booster dose of Tdap; if infected and not previously immunized, antibiotic therapy and immunization started with first dose of toxoid.

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 33 documented cases up to 1976; at least one reported since then; laboratory animal-associated infections have not been reported.

SOURCES/SPECIMENS: Exudates or secretions of the nose, throat (tonsil), pharynx, larynx, wounds; blood, skin

PRIMARY HAZARDS: Inhalation of infectious aerosols and droplets; accidental parenteral inoculation; ingestion

SPECIAL HAZARDS: None
SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for all activities involving known or potentially infected clinical materials or cultures; animal biosafety level 2 facilities for studies utilizing infected laboratory animals

PROTECTIVE CLOTHING: Laboratory coat; gloves when direct contact with infectious materials

OTHER PRECAUTIONS: Administration of adult diphtheria-tetanus toxoid at 10-year intervals to reduce the risk of toxin exposures to laboratory and animal care personnel who work with infected materials

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with absorbent paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal; dispose as chemical waste

STORAGE: In sealed containers that are appropriately labeled. Store in a secure location.

I. RISKS IN LABORATORY WORKERS/CLINICAL SUMMARY

Overview (Portions taken directly from BMBL: http://www.cdc.gov/biosafety/publications/bmbl5/index.htm)

- Diagnostic Tests/Clinical Signs & Symptoms
  Diagnostic testing is not useful in acute management, but blood serum should be obtained and held for later analysis. Clinical signs and symptoms should guide clinical treatment.³

- Pre-exposure Prophylaxis
  A toxoid vaccine is available, and all potentially exposed employees must be fully immunized and receive a booster dose every ten years.

- Post-exposure Prophylaxis/Treatment:
  A Diphtheria antitoxin is available through the CDC for treatment of diphtheria infections, but might be released in the event of exposures in unimmunized individuals, or in the case of exceptionally large exposures in immunized individuals.

II. ADDITIONAL BACKGROUND INFORMATION

DESCRIPTION AND IMPLICATIONS OF RISK:

- Diphtheria is an acute, often fatal, disease caused by the gram-positive bacterium Corynebacterium diphtheriae. C. diphtheriae is transmitted through the air, or in droplets, from human nasopharyngeal secretions, and produces diphtheria toxin, which causes an inflammatory response in the host. Diphtheria toxin affects the respiratory system, causing pseudomembrane formation. The toxin affects protein synthesis once it is present inside target cells. Major target organs of clinical significance include the cardiac, renal and nervous system. Since most of the clinical symptoms of diphtheria are caused by the toxin, prevention focuses on induction of toxin-neutralizing antibodies. The currently available vaccines contain diphtheria toxin treated with formaldehyde (Diphtheria Toxoid).

- The primary diphtheria vaccine series is usually given in a combination injection with tetanus and pertussis vaccines, and is known as the DTP vaccine. A child should have received four DTP shots by 18 months of age, with a booster shot given between the ages of 4 to 6 years. After that, tetanus, diphtheria and pertussis boosters (Tdap) should be given every 10 years to provide continued protection. Several studies have shown that after primary vaccination against diphtheria, in the absence of a natural booster caused by infection with toxigenic strains, anti-toxin antibody concentration showed a continuous fall over 15 years. Twenty five years after primary vaccination, over 20% of vaccinated people would be unprotected. Protective levels of antibody titers are considered greater than 0.001 IU/ml. Fifty percent of diphtheria convalescent patients may have antitoxin titers >3.0 IU/ml (Bissumbhar, et. al).
• Studies show that as age increases, the circulating antibody levels progressively decline. Those greater than 31 years of age show the most significant decline. Occupational risk for diphtheria is increased for non-immunized individuals in close contact with individuals harboring the bacteria and workers handling specimens and cultures containing *C. diphtheriae*. To date, the risk of working with isolated diphtheria toxin is largely unknown. No published data describing the development of diphtheria toxin mediated symptoms, after exposure to the toxin in immunized individuals, has been found.

• According to Mandell’s Principles and Practice of Infectious Diseases, following infection with toxigenic strains of *C. diphtheriae*, or unprotected exposure to diphtheria toxin, subtle evidence of myocarditis can be detected in as many as two thirds of patients approximately one to two weeks after exposure. Cardiac effects may be mild, or involve various types of heart block, arrhythmias, or even congestive heart failure with circulatory collapse. Neurologic symptoms may include paralysis of the soft palate and posterior pharyngeal wall, manifested by regurgitation of swallowed fluids through the nose within the first few days of exposure. Peripheral neuritis may develop from 10 days to three months after exposure.

• In case of unprotected, accidental exposure to toxin, or exposure to toxin that exceeds the protective capacity of neutralizing antibodies in immunized individuals, treatment with hyperimmune antiserum has been shown to reduce mortality from 7 to 2.5 percent. Antibodies only neutralize toxin before its entry into cells, so rapid treatment is essential. Because the diphtheria antiserum (DAT) is produced in horses, up to 10 percent of treated individuals may develop serum sickness. Although diphtheria antitoxin is no longer licensed in the United States, a European-licensed product is available from the National Immunization Program of the CDC by calling 404/639-8200. Information is also available online at: [http://www.cdc.gov/vaccines/vpd-vac/diphtheria/dat/dat-main.htm](http://www.cdc.gov/vaccines/vpd-vac/diphtheria/dat/dat-main.htm)

• “Some of our most valuable information concerning the action of diphtheria toxin in man has been obtained from rare accidents during active immunizations. According to this it appears that the susceptibility of man is per gram weight considerably higher, perhaps ten times higher, than is that of guinea-pigs. The local action of the toxin results in very severe swelling, an inflammatory area surrounded by considerable edema extending both up and down the limb from the point of injection, and even to the trunk. In the center of the inflammatory area sooner or later a large blister filled with sterile serum develops. Finally a considerable slough results. Severe intoxication gives rise to characteristic symptoms consisting of constipation, diminution of urine which may amount to anuria, rapid pulse rate and often considerable mental excitability bordering on hysteria. The temperature is moderately raised. Disturbances of the nerves and muscular system come on early. Diminished power of accommodation of the eye, exaggerated reflexes, followed by diminished knee jerks, and tenderness over the nerve trunks in the neighborhood of the injection can be noted. Action upon the heart is not noted before the ninth day, but from then on is an important factor: it is of the utmost importance for physicians, therefore, to be prepared for late effects upon the heart in diphtheria patients at the time when acute local and general symptoms are subsiding.” (Taken From: *A Textbook of Bacteriology*, Zinsser and Bayne-Jones, 8th Edition, 1939)
IV. ADDITIONAL REFERENCES


² Poisindex


CDC procedures to obtain antitoxin: http://www.cdc.gov/vaccines/vpd-vac/diphtheria/dat/dat-main.htm#how

California Aerosol Transmissible Disease Standard: http://www.dir.ca.gov/Title8/5199.html
