Vanderbilt Occupational Health Protocol for Researchers Using High Dose Diphtheria Toxin

Background:

Diphtheria toxin (DT) is useful in biomedical research using mice, because it can be used to selectively target and kill cells or organs without requiring surgery. Wild type mice do not have DT receptors at all, so they are not affected by DT. The gene for DT receptors can be inserted into a mouse genome so that the transgenic mice will express DT receptors only on specific cells. For example, a transgenic mouse with DT receptors only on hepatocytes can be injected with DT, which will only kill the hepatocytes, creating a nonsurgical mouse model without a functional liver. Some research protocols target transgenic fetal mice in wild type mothers, requiring injecting the mother with extremely high doses of DT to ensure that some crosses the placenta.

Human are very susceptible to DT, which is expressed by strains of Corynebacterium diphtheria which are themselves infected with a bacteriophage that inserts the gene for toxin production. Not all strains of C. diphtheria elaborate toxin; non-toxigenic strains cause milder forms of infection. Toxin elaboration in the throat causes tissue sloughing which is known as trench mouth. If the infection persists, toxin may be expressed distant from the original entry site (usually the mouth/throat) attacks cardiac, nerve and kidney cells among others. The toxin can cause myositis, arrhythmias, neuropathy, paralysis, and kidney failure.

A toxoid vaccine (inactivated toxin) is included in the DT, DTap, Td and Tdap vaccines. Pediatric vaccines with a capital D in the name contain higher doses of toxoid than the adult boosters with a small “d” in the name. An initial series in childhood using DTaP is recommended, followed by boosters with Td every 10 years. At least one adult Td booster should be substituted with Tdap for additional pertussis protection.

Human LD50 for diphtheria toxin is 0.1 ug/kg (7ug for a 70kg person)

Concentration used in mouse research: up to 1.5mg (1500ug) in 0.1-0.3ml (100 – 300 ul) of fluid (Note, 1.5mg is 214 times the human LD50 for a 70 kg person.)

Administrative Controls:

- Toxin stored in locked freezer with access only by approved personnel
- Buddy system – second trained researcher present anytime toxin is in use
- All toxin work happens during business hours on a weekday (non-holiday)
- Mice to be injected are sedated and taped in place
- Injection is done one-handed with immediate activation of the safety feature
- Sharps are disposed of immediately in a sharps box kept at the procedure site

Engineering:

- All reconstitution and dilution happens in a certified biosafety cabinet
- Needle-free system is used for reconstituting toxin from powder
- Safety-engineered sharps used for the mouse injection

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PPE:

- Gloves
- N-95 respirator while transporting lyophilized (freeze dried) toxin in powder form outside of a biosafety cabinet.

Medical Surveillance:

Vaccination:

- Initial series with DTaP if not received in childhood
- Boosters with Td (Tdap if no prior adult Tdap) as indicated by serology

Serologic surveillance:

- Initial antitoxoid must be over 0.1 IU/ml prior to beginning research
  - Note: While the lab calls anything over 0.01 IU/ml "positive", according to CDC diphtheria expert Dr. Tiwari, that level is only minimally protective in majority of people for natural infection. Recommended level for adequate protection is 0.1 IU/ml. Level over 1.0 IU/ml is probably good for 20 years.
- Annual antitoxoid level required
- Td or Tdap booster required if level falls below 0.1 IU/ml
- Additional antitoxoid level checked 4 weeks after each booster

Postexposure Protocol:

- Exposed EE wash site with soap/water or flush (mucous membrane/eye) with water.
- Assistant/buddy secures toxin in locked storage unless spilled, locks lab (If toxin spilled notify HazMat team)
- Exposed EE and assistant proceed to ED with postexposure protocol from lab
- ED initiates postexposure protocol and notifies OHC
  - **Inhalational exposure or accidental injection of toxin** – That level of exposure might warrant deployment of antitoxin. ED to initiate cardiac monitoring, call OHC who will come to ED to coordinate CDC communications. Will notify CDC Emergency Operations Center and speak with Diphtheria Officer. The need for antitoxin to be administered on the CDC's IND protocol will be evaluated case by case.
  - **Needlestick exposure** – OHC will review case with CDC but this would not be an antitoxin deployment situation. Employee should report to ED, ED to call OHC. Get baseline EKG and CPK. Weekly EKG and CPK and physical exam for 3 weeks just to make sure no cardiac or neurologic symptoms.

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Resources:

Resource	Phone	Notes
CDC - Meningitis and Vaccine Preventable Diseases Branch	770-488-7100 Emergency Operations Center 404-639-3158 MPDB office 404-639-8765 Tej Tiwari, SME for diphtheria	Controls access to diphtheria antitoxin, can have us antitoxin in 1 – 3 hours if needed.
Kathryn Edwards		Vanderbilt vaccine expert
Jim Chappell		Vanderbilt Lab director for antitoxoid testing
Drusilla Burns (Deputy Director of CBER at the FDA)	301-402-3553	Recommended vaccine plus serologic surveillance of immunity
James Schmitt (Medical Director of Occ Health at the NIH)	301-496-4411	Hasn't had this concentration of DT used at NIH
John Collier (toxin expert at Harvard)	617-432-1930	
Alan Czarkowski (Medical Director of Occ Health at the NIH)		Hasn't had this concentration of DT used at CDC

FAQs:

1. What booster type to use? **Consensus from Dr. Edwards and CDC is Td/Tdap. Higher dose TD/DTaP not necessary if they had childhood series. More local reactions with higher dose.**
2. Should previously vaccinated personnel be tested for antitoxoid level prior to booster, or just get a fresh booster? **Test for antitoxoid, level >0.1 IU/ml required for research with TD.**
3. How soon after vaccination to test for immunity? **4 weeks based on research protocols studying immune response to toxoid.**
4. How often to screen for immunity? **annually**
5. Given the concentrations of toxin to which people could be exposed, what antitoxoid level would be presumed protective – the usual 0.01 IU/ml or a higher level? **Per Dr. Tiwari at the CDC, level of 0.1 IU/ml is recommended for exposure to toxin at this level.**
6. In the event of accidental exposure e.g. needlestick, how soon might antitoxin be needed (if indicated) and is there time to get this from the CDC? Dr. Dr. Tiwari at the CDC, they could have antitoxin to us in 1 – 3 hours depending upon accessibility from the airport. He thinks neurologic and cardiac effects would take days to weeks even if directly injected with toxin. His advice for exposure management:
    a. **Inhalational exposure or accidental injection of toxin** – That level of exposure might warrant deployment of antitoxin. Employee should proceed to ED, put on cardiac

monitor, call OHC and call CDC Emergency Operations Center to speak with Diphtheria Officer. Will be evaluated case by case.

b. **Needlestick exposure** – Clean the wound, report to ED, call OHC. Get baseline EKG and CPK. OHC can consult with CDC but this would not be an antitoxin deployment situation. Still very low risk. Weekly EKG and CPK and physical exam for 3 weeks just to make sure no cardiac or neurologic symptoms.