

# Advancing Regulatory Science



## CBER-Produced Botulinum Toxoids Support Development of Improved Standards for Testing Botulinum Antitoxin Products

**Highly antigenic botulinum toxoids were used to raise equine antisera for use as standards in tests of botulinum antitoxin products.**

“Characterization of New Formalin-Detoxified Botulinum Neurotoxin Toxoids”

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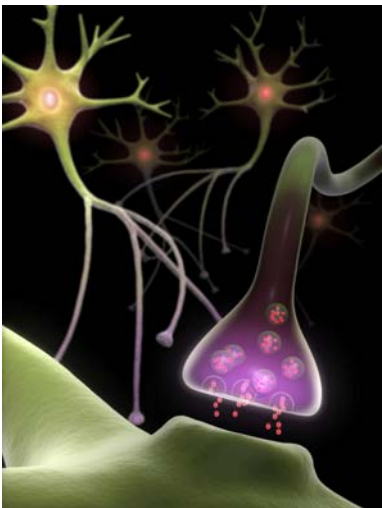
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*Clostridium botulinum*  
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**FDA plays a critical role in the nation’s medical countermeasures (MCM) research and development efforts. The research described here illustrates how CBER regulatory science is enabling the development of safe and effective botulinum antitoxin products and vaccines.**

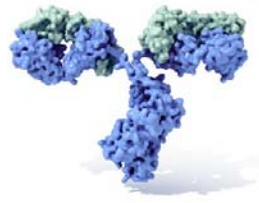
### Reliable Assays Are Needed to Evaluate the Antigenicity of Botulinum Toxoids



- Botulinum neurotoxin (BoNT) blocks nerve activity and causes paralysis.
- The only treatment available after BoNT intoxication is antibody therapy, most often using equine-derived botulinum antitoxin that is made by immunizing horses with formalin-treated BoNT (toxoid).
- An *in vitro* assay that can reliably evaluate the antigenic integrity of botulinum toxoids could track the consistency of the antigen throughout the manufacturing process, identify correlates of potency, and eliminate the need for time-consuming, *in vivo* testing.
- In the future, such an assay could also evaluate the antigenicity of toxoid-based vaccines.

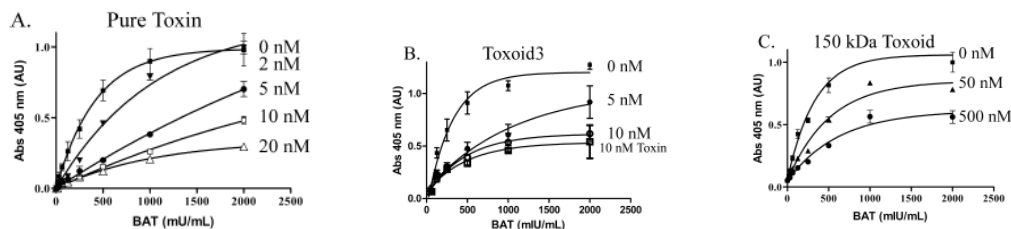
## CBER Develops New Assays to Measure Quality of Antigens Used to Make Antitoxin for Botulinum Toxin Intoxication

- CBER developed inhibition and sandwich enzyme-linked immunosorbent assays (ELISAs) to measure the relative similarity between the toxoid and parent toxin as a quality control test for new botulinum vaccines intended for human use.
- CBER used its ELISAs to monitor the development and refinement of in-house made botulinum toxoids that were subsequently used to raise equine-derived botulism antitoxin.
- The ELISAs enabled same-day assessment of how closely the CBER-produced toxoids mimic the antigenicity of native toxin. This is important because the only alternative test requires *in vivo* immunogenicity measured in animals, which requires many weeks to complete.



### CBER Botulinum Toxoid is Superior to Commercial Toxoid

CBER used its inhibition ELISA to assess the antigenic similarity between native botulinum neurotoxin and both commercial toxoids and the toxoids CBER made using an optimized formalin treatment of native toxin.



- **Graph A.** Native 150-kDa neurotoxin was adsorbed to ELISA plates and detected using serial dilutions of equine antitoxin (30 to 2,000 mU/ml; black boxes).
  - Inhibition analysis was then performed by incubating botulinum antitoxin dilutions with known concentrations of soluble native neurotoxin before adding the dilutions to plates.
  - Soluble neurotoxin in the range of 2 to 20 nM incrementally inhibited botulinum antitoxin binding to the ELISA plate-adsorbed neurotoxin.

- **Graph B.** When the experiment was repeated using CBER-generated toxoid 3, the inhibition pattern was analogous to that caused by native neurotoxin. This suggests that the CBER-produced toxoid 3 is antigenically identical to the native neurotoxin. Toxoid 4 to toxoid 8 performed the same as toxoid 3.
- **Graph C.** Commercially available 150-kDa formalin toxoid at 50 to 500 nM generated partial inhibition of antitoxin binding, indicating this toxoid retained only 2% to 10% antigenic similarity to the native neurotoxin; i.e., epitopes common to the native neurotoxin are 10- to 50-fold less abundant on this commercial toxoid than on the CBER-generated toxoids.
- Three of the CBER-produced toxoids were more than 100,000-fold less toxic in mice than the native BoNT, even though they were antigenically identical to the native toxin as measured by inhibition (ELISA).



Botulinum toxin/Wikimedia Commons

### **CBER Botulinum Toxoids Elicit Highly Protective Antibody Titers in Mice and are Highly Immunogenic in Horses.**

- Mice immunized with toxoids from either of two lots of commercial 150-kDa toxoid or one lot of 900-kDa Hmg toxoid test, failed to survive challenge with 5 LD<sub>50</sub> units of toxin.
- Mice immunized with toxoid 3 or toxoid 8 survived all levels of toxin challenge, which were as high as 275,000 LD<sub>50</sub> units.

**Botulinum toxoids produced by CBER are now used to generate polyclonal antisera that serve as standard assay reagents in inhibition and sandwich ELISAs for testing botulinum antitoxin products.**

**In the future, a botulinum toxoid might be a safe and effective antigen for creating new equine- or human-derived botulinum antitoxin or as a next-generation botulism vaccine for general human use.**