United States Department of Agriculture
Animal and Plant Health Inspection Service
Center for Veterinary Biologics
P. O. Box 844
Ames, IA 50010

ATTENTION:
Select Agent shipping requirements must be met for >1 vial

1. Reagent Name: *Clostridium botulinum* Type C Toxin

2. Strain or Source: Not applicable

3. Lot Number: IRP 421

4. Fill Date: March 18, 1994

5. Expiration Date: No expiration date has been assigned to this product because *C. botulinum* type C toxin has demonstrated over time to be very stable if properly stored. The stability of this reagent will be routinely monitored by the Center for Veterinary Biologics.

Precautions: Exposure to the toxin of *C. botulinum* is a primary hazard. The toxin may be absorbed after ingestion or following contact with the skin, eyes, or mucous membranes, including the respiratory tract.

6. Intended Use: To serve as the standard toxin for potency testing of *C. botulinum* type C bacterin-toxoids. **NOTE: Use SAM 213, Version .02.**

7. Instructions for Use: *C. botulinum* type C toxin IRP 421 diluted 1:40 is considered the standard toxin dilution when conducting potency tests in mink as outlined in title 9, *Code of Federal Regulations* (9 CFR), section 113.110. Prepare the dilution by adding 1.0 mL of IRP 421 to 39.0 mL of sterile 0.067 M phosphate buffered saline (PBS) with 0.2% gelatin, pH 7.2. IRP 421 diluted 1:40 contains $10^{4.0}$ mouse lethal dose fifty (LD_{50}) per 0.5 mL.

8. Test of Reagent:

Determination of LD_{50} - White female mice weighing 16-20 grams were injected in the intraperitoneal cavity with 0.5 mL of toxin diluted in PBS with 0.2% gelatin. The toxin was found to contain $10^{5.711}$ mouse intraperitoneal lethal dose fifty (LD_{50}) per 0.5 mL.

Toxin neutralization test in mice - The toxin test dose was determined in a mouse bioassay by mixing varying amounts of *C. botulinum* type C toxin (IRP 421) with known amounts of *C. botulinum* type C antitoxin, and injecting 0.5 mL of the mixture intraperitoneally into mice. One and five-tenths mL of *C. botulinum* type C International standard antitoxin containing 0.02 units of antitoxin per mL protects 50% of mice when mixed with an equal volume of IRP 421 diluted 1:40,000 in PBS with 0.2% gelatin.

Sterility test - The toxin was tested for sterility and found to be free of viable bacteria and fungi according to procedures outlined in 9 CFR 113.26.

9. Container Size, Type, Weight, or Volume: Five-mL glass vials containing 2.5 mL of toxin.

10. Storage Conditions: Store IRP 421 at -70º± 5°C.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6140 or FAX (515) 337-7673.

12. Origin and Passage History: *C. botulinum* type C (African strain) 203A was used to produce IRP 421. It was obtained in June 1968 from Fromm Laboratories, 703 Lakeshore Road, Grafton, WI 53024.

13. Method of Preparation: Dialysis membranes with a molecular weight cutoff of 12,000-14,000 daltons were filled with PBS and suspended in 1-liter trypsinizing flasks containing proteose peptone-trypticase-yeast extract media with dextrose. The flasks were autoclaved at 121ºC for 25 minutes, cooled, and incubated at 35ºC in an atmosphere containing 5% carbon dioxide, 10% hydrogen, and 85% nitrogen. Approximately 0.5 mL of actively growing culture 203A was aseptically added to the inside of each dialysis membrane and incubated for 120 hours in an anaerobic atmosphere as previously described. The culture was centrifuged at 10,000 x g for 60 minutes. The culture supernatant was adjusted to pH 6.8 and passed through a Millipore filtration unit containing a 0.22-µm membrane. Glycerol was added to a final concentration of 10% volume/volume.

14. Other: <u>Request must be accompanied with a completed APHIS/CDC Form 2.</u>

Reagent orders and feedback should be sent *including phone number* to the following email address: CVB@aphis.usda.gov

Reagent orders forms (APHIS 2018) are available from:
http://www.aphis.usda.gov/animalhealth/cvb_forms

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