United States Department of Agriculture
Animal and Plant Health Inspection Service
Center for Veterinary Biologics
1800 Dayton Avenue
Ames, IA 50010

1. Reagent Name: *Clostridium perfringens* type C (beta) toxin

2. Strain or Source: Not applicable.

3. Lot Number: IRP 513 (04)

4. Fill Date: January 27, 2005

5. Expiration Date: No expiration date has been assigned to this product because *C. perfringens* type C (beta) 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: This reagent does not present a hazard to laboratory personnel who work with the toxin provided good fundamental laboratory techniques are followed.

6. Intended Use: IRP 513 (04) serves as the standard toxin when conducting *C. perfringens* type C toxin-neutralization tests in mice.

7. Instructions for Use: IRP 513 (04) diluted 1:110 is considered the standard toxin dilution when conducting toxin-neutralization tests in mice as outlined in title 9, *Code of Federal Regulations* (9 CFR), section 113.111, and 9 CFR 113.454. The standard toxin dilution is prepared by adding 1.0 mL of IRP 513 (04) to 9.0 mL of peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2). The toxin is further diluted to 1:110 by adding 1.0 mL of the 1:10 dilution to 10.0 mL of diluent. A volume of 0.5 mL of the toxin diluted 1:110 and 0.5 mL of diluent is equivalent to 10 L_o doses. A volume of 0.8 mL of toxin diluted 1:110 and 0.2 mL of diluent is equivalent to 10 L_+ doses. *Clostridium perfringens* type C (beta) toxin IRP 513 (04) diluted 1:10 is stable when stored at -70°± 10°C.

8. Test of Reagent:

Determination of test dose of toxin - The 10 L_o and 10 L_+ doses were established by injecting mice intravenously with 0.2 mL of a mixture of varying amounts of IRP 513 (04) combined with 10 International units (IU) of *C. perfringens* beta antitoxin. The 10 L_o dose for *C. perfringens* type C (beta) toxin neutralization test is the largest amount of toxin which can be mixed with 10 IU of beta antitoxin and not cause death in injected mice within 24 hours. The 10 L_+ dose for *C. perfringens* type C (beta) toxin neutralization test is the smallest amount of toxin which can be mixed with 10 IU of beta antitoxin and cause death in at least 80% of injected mice within 24 hours.

Determination of LD_{50} in mice - Female white Swiss mice weighing 16-20 g were injected intravenously with 0.2 mL of IRP 513 (04) diluted in peptone diluent. The toxin was found to contain $10^{4.016}$ mouse lethal dose fifty (LD_{50}) per 0.2 mL.

Determination of toxin type - The toxin type was confirmed by performing toxin neutralization tests in mice. The mice were injected intravenously with mixtures of IRP 513 (04) and *C. perfringens* type A, B, C, or D antitoxin. All of the mice died within 24 hours except those receiving mixtures containing type B or C antitoxin.

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

9. Container Size, Type, Weight, or Volume: 4.0-mL glass vials containing 1.3 mL of toxin.

10. Storage Conditions: Store at -70°± 10°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. perfringens* type C (beta) culture #4414, used to produce IRP 513 (04), was obtained from Coopers Animal Health, Inc., on July 28, 1975. The number of passages is unknown.

13. Method of Preparation: Culture #4414 was grown in a 14-liter New Brunswick fermentor containing media consisting of N-Z case, proteose peptone, and yeast extract. Actively growing culture was aseptically added to the fermentor and incubated at 35°C for approximately 4 hours. The culture was centrifuged at 10,000 x g for 60 minutes. The supernatant was passed through a Millipore filtration unit containing a 0.2-µm membrane. The filtrate was further processed using a Millipore pellicon cassette system containing a high volume ultrafilter. The concentrated toxin was adjusted to pH 6.6 and passed through a sterile Millipore filtration unit containing a 0.22-mm membrane. Sterile glycerol was added to the product at a final concentration of 15% v/v.

14. Other: None.

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:
https://www.aphis.usda.gov/library/forms/pdf/APHIS_2018.pdf

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