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 692

4. Fill Date: April 1, 2022

5. Expiration Date: October 31, 2027

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 692 serves as the standard toxin when conducting *C. perfringens* type C toxin-neutralization tests in mice.

7. Instructions for Use: IRP 692 diluted 1:35 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 692 to 9.0 mL of peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2). The toxin is further diluted to 1:35 by adding 2.0 mL of the 1:10 dilution to 5.0 mL of diluent. A volume of 0.5 mL of the toxin diluted 1:35 and 0.5 mL of diluent is equivalent to 10 L_o doses. A volume of 0.9 mL of toxin diluted 1:35 and 0.1 mL of diluent is equivalent to 10 L_+ doses. *C. perfringens* type C (beta) toxin IRP 692 diluted 1:10 is stable when stored at -60°C or lower.

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 692 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 >20 g were injected intravenously with 0.2 mL of IRP 692 diluted in peptone diluent. The toxin was found to contain $10^{3.812}$ mouse lethal dose fifty (LD_{50}) per mL.

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: 1.5-mL glass vials containing 0.8 mL of toxin.

10. Storage Conditions: Store at -70°± 10°C.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6100 or FAX (515) 337-7673.

12. Origin and Passage History: *C. perfringens* type C (beta) culture #4414, used to produce IRP 692, 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 10-liter Eppendorf fermenter units containing media consisting of N-Z case, proteose peptone, and yeast extract. Actively growing culture was aseptically added to the fermenter units and incubated at 35°-37°C for approximately 4-5 hours. The culture was centrifuged at 10,000 x g for 45 minutes. The supernatant was passed through Pall Corporation 0.5-µm PreFlow™ DCF and 0.2-µm Supor® DCF membranes. The filtrate was further processed using a Millipore Pellicon® cassette system containing a high-volume ultra-filter. The concentrated toxin was initially passed through a sterile Nalgene filtration unit containing a 0.45-µm membrane followed by filtering through a sterile Nalgene 0.2-µm 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: VS.DB.CVB.Reagent.Requests@usda.gov

Reagent orders forms (APHIS Form 2018) can be found on the CVB website.