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

1. Reagent Name: *Clostridium perfringens* Type A (alpha) toxin

2. Strain or Source: Not applicable.

3. Lot Number: IRP 612

4. Fill Date: April 27, 2012

5. Expiration Date: February 28, 2029

Precautions: This reagent does not present a hazard to laboratory personnel who work with the toxin provided sound fundamental laboratory techniques are followed.

6. Intended Use: IRP 612 serves as the standard toxin when conducting *C. perfringens* Type A (alpha) toxin-neutralization (TN) tests in mice.

7. Instructions for Use: IRP 612 diluted 1:45 is the standard toxin dilution when conducting a 1.0 antitoxin unit per mL (AU/mL) TN test in mice. The toxin dilution is prepared by adding 1.0 mL of well mixed IRP 612 to 9.0 mL of peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2). The toxin is further diluted to 1:45 by adding 4.0 mL of the 1:10 dilution to 14.0 mL of diluent. The L_o dose is prepared by adding 1.0 mL of standard antitoxin containing 1.0 AU/mL to 0.5 mL of standard toxin diluted 1:45 and adjusting the total volume to 2.5 mL with 1.0 mL of diluent. The L_+ dose is prepared by adding 1.0 mL of standard antitoxin containing 1.0 AU/mL to 0.8 mL of standard toxin diluted 1:45 and adjusting the total volume to 2.5 mL with 0.7 mL of diluent.

8. Test of Reagent:

Determination of the test dose of toxin – The L_o dose (largest amount of toxin which, when mixed with 1.0 International unit of antitoxin and injected intravenously into mice, does not cause death within 48 hours) and L_+ dose (smallest amount of toxin which, when mixed with 1.0 International unit of antitoxin and injected intravenously into mice causes death in at least 80% of the mice within 48 hours) were established by injecting 16 to 20 g mice intravenously with 0.5 mL and 0.8 mL volumes of IRP 612 diluted in peptone diluent.

Determination of toxin type – Mice were injected intravenously with a mixture of IRP 612 plus *C. perfringens* Type A antitoxin and IRP 612 plus normal rabbit serum. The mice injected with IRP 612 plus *C. perfringens* type A antitoxin all lived while the mice injected with IRP 612 plus normal rabbit serum all died.

Determination of toxin LD_{50} – Twofold dilutions of IRP 612 were prepared in peptone diluent and 0.5 mL volumes of toxin were injected intravenously into 16 to 20 g mice. The mice were observed for 48 hours and deaths recorded. The toxin lethal dose fifty (LD_{50}) was calculated by the Reed and Muench method and found to contain 3084 LD_{50}/0.50 mL.

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

9. Container Size, Type, Weight, or Volume. Two-mL glass vials containing 1.35 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 A culture 1PA1491/02, obtained from Schering-Plough Animal Health Corporation on April 18, 2003, was used to produce IRP 612. The number of times the culture has been passed is unknown.

13. Method of Preparation: Culture 1PA1491/02 was cultivated in a 14-liter New Brunswick fermentor containing media consisting of proteose peptone, phytone peptone, yeast extract, trypticase peptone, iron sulfate, L-lysine, and zinc sulfate. Eight hundred mL of actively growing culture were aseptically added to the fermentor; the pH adjusted to 7.2 and incubated 5 hours at 35°C. The culture was centrifuged at 10,000 x g for 60 minutes and the supernatant passed through a 0.2-µm Pall Supor® DCF filter. The filtrate was concentrated to one-tenth its original volume using a Millipore pellicon cassette system containing a high-volume ultrafilter. The toxin was passed through a sterile Millipore filtration unit containing a 0.22-µm membrane.

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.