

**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<sub>o</sub> 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<sub>+</sub> 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<sub>o</sub> 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<sub>+</sub> 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<sub>50</sub>* – 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<sub>50</sub>) was calculated by the Reed and Muench method and found to contain 3084 LD<sub>50</sub>/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.

**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 fermenter 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 fermenter; 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](mailto:VS.DB.CVB.Reagent.Requests@usda.gov)

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