

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 D (epsilon) Antitoxin
2. **Strain or Source:** Not applicable
3. **Lot Number:** IRP 249
4. **Fill Date:** December 9, 1981
5. **Expiration Date:** February 28, 2029

**Precautions:** There are no known hazards associated with the use of this reagent.

6. **Intended Use:** To serve as the standard antitoxin when conducting *C. perfringens* type D (epsilon) toxin-neutralization tests in mice.
7. **Instructions for Use:** *Clostridium perfringens* type D (epsilon) antitoxin IRP 249 contains 44 antitoxin units per mL (AU/ml). A dilution of standard antitoxin containing 1.0 AU/mL is used in the toxin-neutralization test as described in title 9, *Code of Federal Regulations* (9 CFR), sections 113.112 and 113.455. The dilution may be prepared by adding 2.0 mL of IRP 249 to 86 mL of peptone diluent. **The 1:44 dilution of antitoxin is stable when stored at  $-70^{\circ}\pm 10^{\circ}\text{C}$  and should be aliquoted for future testing.**

**8. Test of Reagent:**

*Determination of antitoxin titer* - The antitoxin titer was determined by injecting mice with 0.2-mL volumes of diluted antitoxin mixed with  $10\text{ L}_+$  toxin doses (the smallest amount of toxin which, when mixed with 1.0 unit of antitoxin, causes death in at least 80% of the animals within 24 hours) and  $10\text{ L}_0$  toxin doses (the smallest amount of toxin which, when mixed with 1.0 unit of antitoxin, causes no death in animals within 24 hours). The antitoxin titer of IRP 249 was confirmed by injecting mice with toxin-antitoxin mixtures that contained 1.0 mL of IRP 249 diluted 1:44 and mixtures that contained 1.0 mL of *C. perfringens* type D International antitoxin possessing 1.0 unit of antitoxin per mL.

*Sterility test* - IRP 249 was tested for sterility by inoculating it in sterile fluid thioglycollate medium and streaking it on 5% bovine blood agar plates. No detectable growth appeared in the medium or on the blood plates.

**9. Container Size, Type, Weight, or Volume:** Five-mL glass vials each containing 3.4 mL of antitoxin.

**10. Storage Conditions:** Store at -25°C or lower. Once the antitoxin has been thawed, store at 2° to 7°C.

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

**12. Origin and Passage History:** Not applicable

**13. Method of Preparation:** Ponies with no history of clostridial vaccinations received multiple injections of *C. perfringens* type D toxoid and type D toxin during a 12-month period. Serums from the hyper-immunized ponies were fractionated with ammonium sulfate and the immunoglobulin dialyzed against .015 M phosphate buffered saline, pH 6.8. The antitoxin was passed through a Millipore filtration unit containing a 0.22-µm membrane and mixed with 20% sterile glycerol. A final concentration of 1:10,000 Thimerosal was added to the glycerinated antitoxin.

**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.