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 botulinum* type B antitoxin

2. Strain or Source: Not applicable.

3. Lot Number: IRP 435

4. Fill Date: August 11, 1998

5. Expiration Date: No expiration date has been assigned to this product because *C. botulinum* type B antitoxin has demonstrated over time to be very stable if properly stored.

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

6. Intended Use: IRP 435 serves as the standard antitoxin when determining *C. botulinum* type B antitoxin values using a toxin neutralization test in mice.

7. Instructions for Use: IRP 435 contains 5 antitoxin units per mL (AU/mL) when toxin neutralization (TN) tests are conducted in mice at the 0.005 AU/mL level using *C. botulinum* type B toxin IRP 430. Prepare the antitoxin dilutions by transferring 1.0 mL of IRP 435 to 4.0 mL of diluent, transferring 1.0 mL of the 1:5 dilution to 9.0 mL of diluent, transferring 1.0 mL of the 1:50 dilution to 9.0 mL of diluent, transferring 4.0 mL of the 1:500 dilution to 4.0 mL of diluent, and transferring 4.0 mL of the 1:1,000 dilution to 4 mL of diluent (providing test level unit ages of 0.01, 0.005, and 0.0025 AU/mL). Add 1.5 mL of antitoxin to each of three tubes containing 1.5 mL of toxin diluted 1:120,000. Allow the mixture to stand at room temperature for 60 minutes, and inject 5 mice (each weighing 16-20 g) intraperitoneally (i/p) with 0.5 mL of the toxin-antitoxin mixture. Record the number of deaths for 96 hours. Calculate the 50% end point of the standard antitoxin by the Reed and Muench method.

Toxin neutralization tests conducted in mice at the 1.0 AU/mL level using 1.0 L_o and L_+ toxin doses changes the antitoxin titer of IRP 435 to 11 AU/mL. Prepare the antitoxin dilution by transferring 1.0 mL of IRP 435 to 10 mL of diluent. Prepare the L_o dose by adding 2.0 mL of antitoxin, containing 1.0 AU/mL, to 1.0 mL of toxin diluted 1:20 and adjusting the total volume to 4.0 mL with 1.0 mL of diluent. Prepare the L_+ dose by adding 2.0 mL of antitoxin, containing 1.0 AU/mL, to 1.6 mL of toxin diluted 1:20 and adjusting the total volume to 4.0 mL with 0.4 mL of diluent. Allow the mixtures to stand at room temperature for 60 minutes, and inject 5 mice (each weighing 16-20 g) i/p with 0.5 mL of the toxin-antitoxin mixture. Record the number of deaths for 96 hours. All of the mice inoculated with the mixture of antitoxin and L_o dose of toxin must live, and at least 80% of the mice inoculated with the mixture of antitoxin and L_+ dose must die.

Prepare all dilutions in sterile 0.067 M phosphate buffered saline with 0.2% gelatin, pH 7.2.

8. Test of Reagent:

Determination of antitoxin titer – The 0.005 AU/mL level was determined by diluting IRP 435 to an anticipated range of 0.01, 0.005, and 0.0025 AU/mL, and 1.5 mL of each dilution added to a tube

containing 1.5 mL of IRP 430 toxin diluted 1:120,000. The mixture was held at room temperature for 60 minutes and 16-20 g mice were injected i/p with 0.5 mL of the mixture. The antitoxin titer was confirmed by comparing the results of mice injected with toxin-antitoxin mixtures containing 0.01, 0.005, and 0.0025 AU/mL with toxin-antitoxin mixtures containing 0.01 0.005, and 0.0025 International Unit per mL (IU/mL) of *C. botulinum* type B International antitoxin (preparation no. 60/001 received from the National Institute for Biological Standards and Control on May 9, 2000).

The 1.0 AU/mL level was determined by diluting IRP 435 to an anticipated range of 1.0 AU/mL and the antitoxin mixed with 1.0 L_o dose of toxin (the largest amount of toxin which, when mixed with 1.0 unit of antitoxin, causes no deaths in any of the test animals within 96 hours), and 1.0 L_+ dose of toxin (the smallest amount of toxin which, when mixed with 1.0 unit of antitoxin, causes the death of at least 80% of the animals within 96 hours). The mixture was held at room temperature for 60 minutes and 16-20 g mice were injected i/p with 0.5 mL of the mixture. The antitoxin titer was confirmed by comparing the results of mice injected with toxin-antitoxin mixtures containing 1.0 AU/mL with the results of mice injected with toxin-antitoxin mixtures containing 1.0 IU/mL of *C. botulinum* type B International antitoxin (preparation no. 60/001 received from the National Institute for Biological Standards and Control on May 9, 2000).

Sterility test – Five vials of IRP 435 were tested for sterility by inoculating the antitoxin in sterile fluid thioglycollate medium and soybean-casein digest medium. No detectable growth appeared in any tubes of medium.

9. Container Size, Type, Weight, or Volume. Two-mL glass vials containing 1.3 mL of antitoxin.

10. Storage Conditions: Store at -20°C or lower.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6140 or FAX (515) 337-7673.

12. Origin and Passage History: N/A

13. Method of Preparation: Goats with no history of *Clostridial* vaccinations received multiple injections of *C. botulinum* type B toxoid and toxin during a 7 month period. Sera from the hyper-immunized goats were fractionated with ammonium sulfate and the immunoglobulin dialyzed against 0.015 M phosphate buffered saline, pH 7.4. The dialyzed material was passed through a sterile Millipore filtration unit containing a 0.22-µm membrane. No preservatives were added to the product.

14. Other: None

Reagent orders and feedback should be sent *including phone number* to the following email address: VS.STAS.CVB.Reagent.Requests@aphis.usda.gov

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

REVISED: 29Mar18 tlt