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 novyi* Type B Toxin

2. Strain or Source: Not applicable

3. Lot Number: IRP 581

4. Fill Date: April 27, 2009

5. Expiration Date: 20Jul19

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 581 serves as the standard toxin when conducting *C. novyi* alpha toxin-neutralization tests in mice.

7. Instructions for Use: IRP 581 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 113.108. The solution of test toxin is prepared by adding 1.0 mL of well mixed IRP 581 to 9.0 mL of sterile peptone diluent (1.0% peptone, 0.25% sodium chloride, (pH 7.2). The toxin is further diluted to 1:35 by adding 1.0 mL of the 1:10 dilution to 2.5 mL of peptone diluent. A volume of 0.5 mL of the toxin diluted 1:35 and 0.5 mL of diluent is equivalent to 0.1 L_o dose. A volume of 0.8 mL of toxin and 0.2 mL of diluent is equivalent to 0.1 L_+ dose.

8. Test of Reagent:

Determination of test dose of toxin - The 0.1 L_o and 0.l L_+ doses were established by injecting mice intravenously with 0.2 ml of a mixture of varying amounts of IRP 581 combined with 0.1 International Unit of antitoxin. The 0.1 L_o dose for the *C. novyi* type B toxin neutralization test is the largest amount of toxin which can be mixed with one-tenth unit of Standard Antitoxin and not cause death in injected mice within 72 hours. The 0.1 L_+ dose for the *C. novyi* type B toxin neutralization test is the smallest amount of toxin which can be mixed with one-tenth unit of Standard Antitoxin and cause death in at least 80% of injected mice within 72 hours.

Determination of toxin type - Toxicity studies in mice demonstrated that the lethal effect of *C. novyi* type B toxin IRP 581 was neutralized by *C. novyi* type A (alpha) antitoxin. An *in vitro* neutralization of the lecithovitellin reaction by *C. novyi* type B (beta) antitoxin was used to help confirm toxin identity. Test results showed that IRP 581 contains no detectable beta toxin.

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

9. Container Size, Type, Weight, or Volume: Five-mL glass vials containing 1.3 mL of toxin.

10. Storage Conditions: Store at -70°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: *C. novyi* type B strain CN 234.3 (IRP 190), used to produce IRP 581, was obtained from Wellcome Research Laboratories, Beckenham, England, on July 16, 1965. The number of passages is unknown.

13. Method of Preparation: Culture CN 234.3 (IRP 190) was grown in dialysis membranes with a molecular weight cutoff range from 12,000 to 14,000 daltons. The membranes were filled with 0.15 M phosphate buffered saline, pH 7.4, and suspended in 1-liter trypsinizing flasks containing media consisting of proteose peptone, trypticase, yeast extract, and liver powder. Actively growing culture was aseptically added to the inside of the dialysis membranes and incubated at 35ºC for 120 hours in an anaerobic glove box containing 85% nitrogen (N), 10% hydrogen (H), and 5% carbon dioxide (CO). The culture was centrifuged at 10,000 x g for 70 minutes. The culture supernatant was passed through a sterile Corning 150-mL bottle top filter containing a 0.22-µm membrane.

14. Other: None

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

Reagent orders forms (APHIS 2018) are available from:
https://www.aphis.usda.gov/library/forms/pdf/APHIS_2018.pdf

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