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 septicum* (alpha) Toxin

2. Strain or Source: Not applicable.

3. Lot Number: IRP 455

4. Fill Date: October 13, 2000

5. Expiration Date: No expiration date has been assigned to this product because *C. septicum* alpha toxin has demonstrated over time to be very stable if properly stored. The stability of this reagent will be routinely monitored by the Bacteriology Laboratory, Center for Veterinary Biologics.

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

6. Intended Use: IRP 455 serves as the standard toxin when conducting *C. septicum* alpha toxin-neutralization (TN) test in mice.

7. Instructions for Use: IRP 455 diluted 1:40 is considered the standard toxin dilution when conducting TN tests in mice. The toxin dilution is prepared by adding 1.0 mL of well mixed IRP 455 to 39.0 mL of sterile peptone diluent (1% peptone, 0.25% sodium chloride, pH 7.2).

The L_o dose is prepared by adding 2.0 mL of standard antitoxin (1.0 antitoxin unit per mL [AU/mL)]) to a tube containing 1.0 mL of standard toxin (diluted 1:40) and 2.0 mL of diluent.

The L_+ dose is prepared by adding 2.0 mL of standard antitoxin (containing 1.0 AU/mL) to a tube containing 1.6 mL of standard toxin (diluted 1:40) and 1.4 mL of diluent.

8. Test of Reagent:

Determination of LD_{50} - Harlan Sprague Dawley female mice weighing 16-20 g were injected intravenously with 0.5 mL of toxin diluted in peptone diluent. The toxin was found to contain 1200 mouse lethal dose fifty (LD_{50}) per 0.5 mL.

Determination of test dose of toxin - The L_o and L_+ doses were established by injecting mice intravenously with 0.5 mL of a mixture containing varying amounts of IRP 455 combined with

1.0 International Unit (IU) of *C. septicum* alpha antitoxin. The L_o dose for the TN test is the largest amount of toxin which can be mixed with 1.0 IU of antitoxin and not cause death in injected mice within 72 hours. The L_+ dose for the TN test is the smallest amount of toxin which can be mixed with 1.0 IU of antitoxin and cause death in at least 80% of injected mice within 72 hours.

Sterility test – Five vials of IRP 455 were tested for sterility by inoculating the contents of each vial into sterile fluid thioglycollate medium and soybean-casein digest medium. All 5 vials of toxin were free of bacteria and fungi.

9. Container Size, Type, Weight, or Volume: Three-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. septicum* strain CN3204, used to produce IRP 455, was obtained from Wellcome Research Laboratories, Beckenham, England, on June 29, 1977. The number of passages is unknown.

13. Method of Preparation: Strain CN3204 was grown in a 14-liter New Brunswick fermentor containing media consisting of proteose peptone, trypticase, and yeast extract. Actively growing culture was aseptically added to the fermentor and incubated at 35°C for 13 hours. The culture was centrifuged at 10,000 x g for 60 minutes and the supernatant passed through a Millipore filtration unit containing a 0.22-µm membrane. The filtrate was further processed using a Millipore pellicon cassette system containing a high volume ultrafilter. The solution retained by the ultrafilter was adjusted to pH 6.7 and 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: CVB@aphis.usda.gov

Reagent orders forms (APHIS 2018) are available from:
http://www.aphis.usda.gov/animalhealth/cvb_forms

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