

**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 688
4. **Fill Date:** November 19, 2021
5. **Expiration Date:** February 28, 2029

**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 688 serves as the standard toxin when conducting *C. septicum* alpha toxin-neutralization (TN) test in mice.

7. **Instructions for Use:**

*Mouse assay:* IRP 688 diluted 1:20 is considered the standard toxin dilution when conducting toxin neutralization tests in mice. The toxin dilution is prepared by adding 1.0 mL of well mixed IRP 688 to 19.0 mL of sterile peptone diluent (1% peptone, 0.25% sodium chloride, pH 7.2). The L<sub>o</sub> 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 1:20) and 2.0 mL of diluent. The L<sub>+</sub> 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 1:20) and 1.4 mL of diluent.

8. **Test of Reagent:**

*Determination of LD<sub>50</sub>* – Swiss Webster female mice weighing 16-20 g were injected intravenously with toxin diluted in peptone diluent and LD<sub>50</sub> calculations performed by the method of Reed and Muench. The toxin was determined to contain one mouse lethal dose fifty (LD<sub>50</sub>) per 0.5 mL of a 1:830 dilution.

*Determination of test dose of toxin* – The L<sub>o</sub> and L<sub>+</sub> doses were established by injecting mice intravenously with 0.5 mL of a mixture containing varying amounts of IRP 688 combined with 1.0 International Unit (IU) of *C. septicum* alpha antitoxin. The L<sub>o</sub> 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<sub>+</sub> 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* - Ten vials of IRP 688 were tested for sterility by inoculating the contents of each vial into sterile fluid thioglycollate medium with beef and soybean-casein digest medium. All 10 vials of toxin were free of bacteria and fungi.

**9. Container Size, Type, Weight, or Volume:** Four-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) 337-6100 or FAX (515) 337-7673.

**12. Origin and Passage History:** *C. septicum* strain CN3204, used to produce IRP 688, 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 two 10-liter Bioflo fermentors containing media consisting of Bacto Peptone, Trypticase Peptone, and Yeast Extract. Actively growing culture was aseptically added to the fermentor and incubated at 35°C for 14 hours. The culture was centrifuged at 10,000 x g for 45 minutes and the supernatant passed through a Pall Preflow™ DCF 0.5-µm filter and then passed through a Pall Supor® DCF filtration unit containing a 0.2-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 passed through sterile Nalgene SFCA filtration units containing 0.45-µm and 0.2-µm membranes.

**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.