

**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 628
4. **Fill Date:** 29Aug13
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 628 serves as the standard toxin when conducting *C. septicum* alpha toxin-neutralization (TN) test in mice.

7. **Instructions for Use:**

Mouse assay: IRP 628 diluted 1:20 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 628 to 19.0 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 1:20) 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 1:20) and 1.4 mL of diluent.

VERO cell assay (Final Product): IRP 628 diluted to 1:60 is considered the standard toxin dilution when conducting the VERO cell assay as outlined in BBPRO1009. The dilution is prepared by adding 1.0 mL of IRP 628 to 5.0 mL of Minimal Essential Medium (MEM) with Earles F-15 with 0.5% LAH, containing 1% L-glutamine, 0.1% pen-strep, and 5% fetal bovine serum. The toxin is further diluted to 1:60 by adding 0.5 mL of 1:6 dilution to 4.5 mL of diluent; this dilution is considered the standard toxin preparation and is used in the toxin control wells.

Dilute the standard toxin preparation further according to the following table:

Tube #	Microliters of 1:60 toxin	DMEM in mL	Final dilution
1	222	2	1:600
2	245	2	1:550
3	273	2	1:500
4	308	2	1:450
5	353	2	1:400

Add the graduated toxin to each column according to the following template:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		Toxin diluted 1:600	Toxin diluted 1:550	Toxin diluted 1:500	Toxin diluted 1:450	Toxin diluted 1:400	Live Control	Toxin diluted to 1:550	Toxin diluted to 1:500	Toxin diluted to 1:450	Toxin diluted to 1:400	
C												
D												
E												
F												
G												
H												

8. Test of Reagent:

Determination of LD₅₀ - Harlan Sprague Dawley female mice weighing 16-22 g were injected intravenously with 0.5 mL of toxin diluted in peptone diluent. The toxin was found to contain 536 mouse lethal dose fifty (LD₅₀) per 0.5 mL.

Determination of test dose of toxin - The L₀ and L₊ doses were established by injecting mice intravenously with 0.5 mL of a mixture containing varying amounts of IRP 628 combined with 1.0 International Unit (IU) of *C. septicum* alpha antitoxin. The L₀ 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 - Ten vials of IRP 628 were tested for sterility by inoculating the contents of each vial into sterile fluid thioglycollate medium and soybean-casein digest medium. All 10 vials of toxin were free of bacteria and fungi.

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. septicum* strain CN3204, used to produce IRP 628, 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:
https://www.aphis.usda.gov/library/forms/pdf/APHIS_2018.pdf

REVISED: 03Dec15 alb