

**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* Cell Assay Proficiency Panel
2. **Strain or Source:** NA
3. **Lot Number:** IRP 639
4. **Fill Date:** 23Jun16
5. **Expiration Date:** Not Applicable

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 639 for use in the VERO cell line assay test as described in **BBPRO1009** for proficiency testing.
7. **Composition of Proficiency Panel:** The IRP 639 panel contains 24 vials of the Standard Toxin at the working stock dilution of 1:10, 24 vials of the Standard Antitoxin at the working stock dilution of 10 AU/mL, and 24 vials of rabbit serum samples.
8. **Instructions for Use:** At least one individual at the participating laboratories is required to run one entire panel. Testing should be completed as described in **CVB-PRO-0009**. Copies of **BBPRO1009** are available upon request and are included with each panel.

The Standard Antitoxin is diluted to 0.1 AU/mL by adding 1.0 mL of the 10 AU/mL working stock dilution to 9.0 mL of DMEM diluent, and then further diluting to 0.1 AU/mL by adding 1.0 mL of the 1 AU/mL dilution to 9.0 mL of DMEM diluent.

Rabbit serum samples are diluted 1:10 by adding 300µL to 2.7 mL DMEM diluent.

The Standard Toxin is further diluted to 1:60 by adding 1.0 mL of the 1:10 working stock dilution to 5.0 mL of DMEM diluent. 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 cell Control	Toxin diluted to 1:550	Toxin diluted to 1:500	Toxin diluted to 1:450	Toxin diluted to 1:400					
C																
D							Toxin Control									
E																
F																
G																
H																

9. Test of Reagent: The rabbit serum samples were first identified by using an endpoint cell assay to determine the AU/mL level in each. A varying number of passing and failing serums are provided.

10. Container Size, Type, Weight, or Volume: All vials are supplied in an 81 vial square box. The Standard Toxin vials are 1.5 mL screw cap plastic vials containing 1.3 mL/vial; the Standard Antitoxin vials are 1.5 mL screw cap plastic vials containing 1.3 mL/vial; the rabbit serum samples are 0.5 mL screw cap vials containing 350 µL/vial.

11. Submitting Test results: Please submit the test results to Bacteriology Section, APHIS-CVBPELBACTLabVMOMicros@usda.gov.

CVB-DAT-0139.02

Page 2 of 3

12. Laboratory pass/fail criteria: For each sample, a result is considered passing based on comparing the results to those obtained from the CVB lab.

13. Reporting Laboratory test scores: Participating laboratories are required to run at least one proficiency panel and provide those results to the CVB to receive approval prior to evaluating their products and submitting changes to their approved Outlines of Production.

14. Storage Conditions: Store at -60°C or lower.

15. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6100.

16. Origin and Passage History:

Standard Toxin – *C. septicum* toxin (IRP 628)

Standard Antitoxin – *C. septicum* Antitoxin (IRP 600)

Rabbit serum samples were obtained from New Zealand White rabbits vaccinated with products containing *C. septicum* antigen.

17. Method of Preparation: The Standard Toxin was previously prepared (see the Reagent Data Sheet for IRP 628). The Standard Antitoxin was previously prepared (see the Reagent Data Sheet for IRP 600). The rabbit serums samples were produced according to CVB-PRO-0010. The blood tubes were centrifuged at 1200 RPM. The serum was poured off, filtered through a 0.22 µm Millipore syringe filter and aliquoted.

18. Other:

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

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