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* Antitoxin

2. Strain or Source: Not applicable.

3. Lot Number: IRP 228

4. Fill Date: June 30, 1976

5. Expiration Date: No expiration date has been assigned to this product because *C. septicum* alpha antitoxin 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 antitoxin provided standard laboratory practices are followed.

6. Intended Use: IRP 228 serves as the standard antitoxin when determining *C. septicum* alpha antitoxin values using a toxin neutralization test in mice.

7. Instructions for Use: *C. septicum* antitoxin IRP 228 contains 960 units of antitoxin per vial. One mL of standard antitoxin containing 1.0 antitoxin unit per mL (AU/mL) is used when determining the antitoxin content of serum from animals vaccinated with product containing *C. septicum toxoid.*

Rehydrate the standard antitoxin by adding 5.0 mL of peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2) to a vial of IRP 228 and placing it at 2°- 7°C for 16-24 hours. Transfer the reconstituted antitoxin to a tube. Wash the vial with 15.0 mL of diluent using 5.0 mL of diluent per wash. Add an additional 28.0 mL of diluent to the tube and thoroughly mix the contents. The antitoxin mixture containing 20 AU/mL should be dispensed into individual tubes and stored at -70°C or lower.

Add 1.0 mL of antitoxin (containing 20 AU/mL) to 19.0 mL of diluent to obtain a solution containing 1.0 AU/mL.

8. Test of Reagent:

Determination of antitoxin titer - The antitoxin titer of IRP 228 was determined by injecting mice intravenously with 0.5 mL of diluted antitoxin mixed with 1.0 L_+ dose of toxin (the smallest amount of toxin which, when mixed with 1.0 unit of antitoxin, causes death in at least

80% of the animals within 72 hours) and 1.0 L_o dose of toxin (the largest amount of toxin which, when mixed with 1.0 unit of antitoxin, causes no deaths in any of the animals within 72 hours). The antitoxin titer was confirmed by comparing the results of mice injected with toxin-antitoxin mixtures containing 1.0 mL of IRP 228 possessing 1.0 unit of antitoxin to the results of mice injected with toxin-antitoxin mixtures containing 1.0 mL of *C. septicum* International antitoxin possessing 1.0 AU/mL.

Sterility test – Four vials of IRP 228 were tested for sterility by inoculating the contents of each vial into sterile fluid thioglycollate medium and soybean-casein digest medium. No detectable growth appeared in any tubes of medium.

9. Container Size, Type, Weight, or Volume: Ten-mL glass vials containing 2.0 mL of lyophilized antitoxin.

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: Not applicable.

13. Method of Preparation: Two-year-old ponies weighing 400 to 500 pounds with no history of clostridial vaccinations received multiple injections of *C. septicum* toxoid and toxin during a 7-month period. Serum collected from the hyperimmunized animals was fractionated with ammonium sulfate and the immunoglobulin dialyzed against 0.01 M phosphate buffered saline, pH 7.4. The dialyzed material was passed through a sterile Millipore filtration unit containing a 0.22-µm membrane. Thimerosal was added to the product at a final concentration of 0.01%. The ponies used for antitoxin preparation tested negative on serological tests for leptospirosis and brucellosis.

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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