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 sordellii* Toxin

2. Strain or Source: Not Applicable

3. Lot Number: IRP 675

4. Fill Date: November 7, 2019

5. Expiration Date: February 28, 2029

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

6. Intended Use: IRP 675 serves as the standard toxin when conducting *C. sordellii* toxin-neutralization (TN) tests in mice.

7. Instructions for Use: *C. sordellii* toxin IRP 675 diluted 1:10 is considered the standard toxin dilution when conducting TN tests in mice as outlined in title 9, *Code of Federal Regulations* (9 CFR), part 113.109. The standard toxin dilution is prepared by adding 1.0 mL of well mixed IRP 675 to 9.0 mL of sterile peptone diluent (1.0% peptone, 0.25% sodium chloride, pH 7.2). A volume of 0.5 mL of the toxin diluted 1:10 and 0.5 mL of diluent represents 1.0 L_o toxin dose. A volume of 0.8 mL of the toxin diluted 1:10 and 0.2 mL of diluent represents 1.0 L_+ dose.

8. Test of Reagent: *Determination of test dose of toxin* – The L_o and L_+ doses were established by injecting 16 to 20 gram mice intravenously with 0.2 mL of varying amounts of IRP 675 combined with 1.0 mL of *C. sordellii* antitoxin IRP 501 containing 1.0 antitoxin unit per mL (AU/mL). The L_o and L_+ doses were confirmed by injecting 16 to 20 gram mice intravenously with 0.2 mL of varying amounts of IRP 675 combined with 1.0 mL of International Antitoxin containing 1.0 IU/mL.

The L_o dose for the *C. sordellii* TN test is the largest quantity of toxin which can be mixed with 1.0 unit of antitoxin and not cause death in injected mice within 72 hours. The L_+ dose for the *C. sordellii* TN test is the smallest amount of toxin which can be mixed with 1.0 unit of antitoxin and cause death in at least 80% of injected mice within 72 hours.

Determination of LD_{50} – White Swiss mice weighing 16-20 g were injected intravenously with 0.2 mL of IRP 675 diluted in peptone diluent. The toxin was found to contain $10^{4.5}$ lethal dose fifty (LD_{50}) per 0.2 mL.

Sterility test – The toxin was tested for sterility and found to be free of viable bacteria and fungi according to procedures outlined in 9 CFR 113.26.

9. Container Size, Type, Weight, or Volume: 4-mL glass vials containing 1.3 mL of toxin

10. Storage Conditions: Store at -50° to -90°C.

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

12. Origin and Passage History: *C. sordellii* culture No. 7502, used to produce IRP 675, was obtained September 16, 1968, from Montana State University, Bozeman, Montana. The number of passages is unknown.

13. Method of Preparation: Culture 7502-1-11 was grown in dialysis membranes with a molecular weight cutoff range from 12,000 to 14,000 daltons. The membranes were filled with 0.15 M phosphate buffered saline, pH 7.4, and suspended in 1-liter trypsinizing flasks containing media consisting of Brain Heart Infusion Broth. Actively growing culture was aseptically added to the inside of the dialysis membranes and incubated at 35ºC for 50 hours in an anaerobic glove box containing 85% nitrogen (N), 10% hydrogen (H), and 5% carbon dioxide (CO). The culture was centrifuged at 10,000 x g for 60 minutes. The culture supernatant was passed through a sterile Pall VacuCap 90 PF filter unit containing a 0.8/0.2-µm Supor® membrane.

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.

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