Clostridium sordellii Toxin - IRP 675

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Notes:
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:** December 31, 2024

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

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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 L0 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 L0 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 L0 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 L0 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<sub>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<sup>4.5</sup> lethal dose fifty (LD<sub>50</sub>) 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) 337-7020 or FAX (515) 337-7673

12. **Origin and Passage History:** *C. sordelli* 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 35°C 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.