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 tetani* Toxin

2. Strain or Source: Not applicable

3. Lot Number: IRP 648

4. Fill Date: May 25, 2017

5. Expiration Date: 30Nov20

Precautions: Accidental parenteral inoculation and ingestion of the toxin are the primary hazards associated with this reagent. It is uncertain if tetanus toxin can be absorbed through mucous membranes; consequently, the hazards associated with aerosols and droplets remain unclear. The administration of an adult diphtheria-tetanus toxoid at 10-year intervals reduces the risk of toxin exposures to laboratory personnel and is highly recommended.

6. Intended Use: Use IRP 648 to coat microtiter plates for the indirect enzyme-linked immunosorbent assay (ELISA) as described in **SAM 217**, and for the comparative toxin-antitoxin neutralization test in guinea pigs as described in **SAM 206**.

7. Instructions for Use:

9CFR 113.217 Tetanus Toxoid: To conduct ELISA, coat each well of the microtiter plate with 100 µL of IRP 648 diluted 1:12 by adding 1 mL IRP 648 + 11 mL antigen coating buffer (ACB) or 2 mL IRP 648 + 22 mL ACB (for two plates) in antigen coating buffer.

9CFR 113.451 Tetanus Antitoxin: To conduct comparative toxin-antitoxin neutralization tests in guinea pigs at the 0.10 Antitoxin Unit per mL level, dilute IRP 648 1:825, in 1/15 M phosphate buffered saline, pH 7.4, with 0.2% gelatin (PBS w/gelatin) by adding 1 mL IRP 648 + 99 mL PBS w/ gelatin (1:100); 2 mL (1:100) + 14.5 mL PBS w/ gelatin.

8. Test of Reagent: *Determination of the test dose of toxin* – Titrations were performed to determine the optimum toxin concentration for adsorption to 96-well microtiter plates.

Sterility Test - The toxin was tested for sterility and found to be free of viable bacteria and fungi according to 9 CFR 113.26.

9. Container Size, Type, Weight, or Volume: 4.0-mL screw-cap vials containing 2.3 mL of toxin.

10. Storage Conditions: Store IRP 648 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: *Clostridium tetani* culture 7010 obtained from Burns Bio-tech was used to produce IRP 648. The history of the culture prior to being sent to the Center for Veterinary Biologics is unknown.

13. Method of Preparation: The culture was grown in a 14-liter fermentor vessel containing modified Mueller and Miller medium. The culture was incubated at 35°- 36°C for 110-120 hours. The culture was centrifuged at 10,000 x g in a rotor chamber temperature of 2º- 7°C for 45 minutes. The supernatant was passed through a 0.2-µm Nalgene™ Rapid Flow™ vacuum PES filter. The filtrate was concentrated using a Millipore pellicon cassette system containing a 10,000 NMWL PTGC00005 filter. The material retained by the filter was passed through a sterile 0.2-µm Nalgene™ Rapid Flow™ vacuum PES filter.

14. Other: None

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

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

REVISED: 04May18 tlt