Animal and Plant Health Inspection Service

Veterinary Services

Center for Veterinary Biologics

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Clostridium haemolyticum spore preparation - Lot ID IRP 526 (06)

Document Number: **CVB-DAT-0095** **Revision:** **03**

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Effective Date: 28 Oct 2020

Notes: *Strain or Source: N/A, Fill date: August 23, 2006*

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 haemolyticum* spore preparation

2. **Strain or Source:** Not applicable

3. **Lot Number:** IRP 526 (06)

4. **Fill Date:** August 23, 2006

5. **Expiration Date:** August 31, 2025

Precautions: Personnel must take precautions against being stuck with needles or cut with sharp instruments contaminated with *C. haemolyticum* spores.

6. **Intended Use:** For use in challenging the immunity of guinea pigs vaccinated with *C. haemolyticum* containing products.

7. **Instructions for Use:** Guinea pigs used to test the potency of biological products containing *C. haemolyticum* are challenged as outlined in title 9, *Code of Federal Regulations* (9 CFR), section 113.107. The challenge dose is prepared by adding 0.5 mL of well mixed IRP 526 (06) to 4.5 mL of sterile saline (0.85% NaCl) solution. The spore suspension is further diluted by adding 1.0 mL of well mixed 1:10 dilution to 9.0 mL of saline solution. The 1:4,000 challenge dilution is prepared by adding 1.0 mL of well mixed 1:100 dilution to 39 mL of 7.5% calcium chloride ($CaCl_2 \cdot 2H_2O$) solution. The challenge dilution should be prepared 10-15 minutes before the test animals are inoculated. The challenge dose is 0.5 mL administered by intramuscular injection.

8. **Test of Reagent:**

Determination of lethal dose fifty (LD_{50}) in guinea pigs - The LD_{50} was determined by injecting 300 to 500 gram guinea pigs with twofold dilutions of IRP 526 (06). The weighted mean LD_{50} was calculated from the test results by fitted regression curve analysis (95% CI) and the mean LD_{50} was calculated by the method of Reed and Muench. The LD_{50} was found to be 1:400,000 per 0.5 mL of diluted spore suspension.

Purity test - 526 (06) was tested for purity and found to be a pure culture of *C. haemolyticum* based on cellular and colony morphology and with no growth of extraneous bacteria or fungi.

9. **Container Size, Type, Weight, or Volume:** Two-mL glass vials containing 0.8 mL of spore suspension.

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10. Storage Conditions: Store IRP 526 (06) at -70°± 10°C. Repeated freezing and thawing is not recommended.

11. CVB Technical Contact: Center for Veterinary Biologics, Bacteriology Section, (515) 337-6140 or FAX (515) 337-7673.

12. Origin and Passage History: IRP 526 (06) was prepared from *C. haemolyticum* IRP 89. The culture was originally isolated from the liver of a cow which died of bacillary hemoglobinuria. The culture, identified as strain 7170, was obtained from Montana State University, Bozeman, Montana, in 1962. The number of passages is unknown.

13. Method of Preparation: *C. haemolyticum* spores were cultivated on the surface of liver-digest agar medium in 500-mL Erlenmeyer flasks. The flasks were incubated at 35°C for 5 days in an anaerobic glove box. The spores were washed from the agar surface with sterile phosphate buffer and suspended in an equal volume of sterile glycerol.

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.