

**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 647
4. **Fill Date:** July 22, 2020
5. **Expiration Date:** February 28, 2029

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 *Clostridium 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 647 to 4.5 mL of sterile saline (0.85% NaCl) solution. The 1:140 challenge dilution is prepared by adding 2.0 mL of well mixed 1:10 dilution to 26.0 mL of 7.5% calcium chloride (CaCl₂·2H₂O) 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 (LD₅₀) in guinea pigs – 300 to 500-gram guinea pigs were injected with twofold dilutions of IRP 647. For each test, the LD₅₀ was calculated by the method of Reed and Muench. The average of these calculations determined 1 LD₅₀ is contained in 0.5 mL of a 1:14,000 dilution of the spore suspension.

Purity test – IRP 647 was tested for purity according to 9CFR 113.27 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.

10. Storage Conditions: Store IRP 647 at $-80^{\circ}\pm 10^{\circ}\text{C}$. Repeated freezing and thawing is not recommended.

11. CVB Technical Contact: Center for Veterinary Biologics, Bacteriology Section, (515) 337-6100 or FAX (515) 337-7673.

12. Origin and Passage History: IRP 647 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

Restrictions: N/A

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.