

**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 chauvoei* Spore Preparation
2. **Strain or Source:** Not Applicable
3. **Lot Number:** IRP 631
4. **Fill Date:** October 16, 2015
5. **Expiration Date:** February 28, 2029

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

6. **Intended Use:** IRP 631 serves as the standard challenge material for use in evaluating the potency of biological products containing *C. chauvoei*.

7. **Instructions for Use:** IRP 631 diluted 1:37,000 is considered the challenge dilution for conducting *C. chauvoei* potency tests in guinea pigs as outlined in title 9, *Code of Federal Regulations* (9 CFR), section 113.106. A 1:100 dilution is prepared by adding 1.0 mL of well mixed IRP 631 to 99.0 mL of sterile 0.85% sodium chloride (NaCl) solution. The spore suspension is further diluted to 1:3700 by adding 1.0 mL of the diluted spore suspension (1:100) into 36.0 mL of sterile 0.85% NaCl solution. The final challenge dilution (1:37,000) is prepared by adding 3.0 mL of well-mixed 1:3700 dilution to 27 mL of 7.5% calcium chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) solution.

8. **Test of Reagent:**

*Determination of culture purity* – IRP 631 was tested for purity and found to be a pure culture of *C. chauvoei* based on 9 CFR 113.27 testing.

*Determination of spore preparation LD<sub>50</sub>* – Guinea pigs weighing 500 to 600 grams were injected intramuscularly with 0.5 mL of IRP 631 diluted in 7.5%  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  solution. The guinea pig LD<sub>50</sub> was calculated by the method of Reed and Muench and found to be 1:3,680,000 per 0.5 mL of spore suspension.

9. **Container Size, Type, Weight, or Volume:** Five-mL glass vials containing 1.5 mL of spore suspension.

10. **Storage Conditions:** Store at <-60°C.

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

**12. Origin and Passage History:** *Clostridium chauvoei* IRP 631 was prepared from *Clostridium chauvoei* IRP 206, which was prepared from *C. chauvoei* Lot F obtained from the American Cyanamid Company in 1962. The number of times the culture was passed prior to being sent to the Center for Veterinary Biologics was unknown.

**13. Method of Preparation:** *Clostridium chauvoei* spores were cultivated on the surface of a beef infusion agar medium in 500-mL Erlenmeyer flasks. The flasks were incubated in an anaerobic chamber containing 85% nitrogen (N<sub>2</sub>), 10% hydrogen (H<sub>2</sub>), and 5% carbon dioxide (CO<sub>2</sub>) at 35°C for 2 to 4 days than 25°- 29°C for 4 days before spores were harvested by washing the agar surface with sterile 0.015 M phosphate buffered saline, pH 6.9. The spore suspension was then mixed with 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](mailto:VS.DB.CVB.Reagent.Requests@usda.gov).

Reagent order forms (APHIS Form 2018) can be found on the Biologics Forms page of the CVB website.