



Animal and Plant
Health Inspection
Service

Veterinary Services

Center for Veterinary
Biologics

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Clostridium chauvoei Spore Preparation - Lot ID IRP 631

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Notes: *Strain or Source: N/A, Fill date: 16Oct15*

**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:** December 31, 2024

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 LD₅₀ – 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 LD₅₀ 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) 337-7020 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 (N₂), 10% hydrogen (H₂), and 5% carbon dioxide (CO₂) 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.

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