

**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 sordellii* Challenge Culture
2. **Strain or Source:** Not applicable.
3. **Lot Number:** IRP 313
4. **Fill Date:** December 12, 1985
5. **Expiration Date:** No expiration date has been assigned to this product because *C. sordellii* spores have demonstrated over time to be very stable if properly stored. This reagent will be monitored by the Center for Veterinary Biologics.

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

6. **Intended Use:** IRP 313 can be used to challenge the immunity of guinea pigs vaccinated with biological products containing *C. sordellii* toxoids or bacterin-toxoids.

7. **Instructions for Use:** Guinea pigs immunized with *C. sordellii*-containing products are injected intramuscularly with 0.5 mL of IRP 313 diluted 1:15,000 in 5% calcium chloride (CaCl₂·2H₂O) solution. The challenge dilution is prepared by adding 1.0 mL of well mixed IRP 313 to 14 mL of sterile saline solution (0.85% sodium chloride). The spore suspension is further diluted by adding 1.0 mL of the 1:15 dilution to 99.0 mL of saline solution. The final challenge dilution is prepared by adding 3.0 mL of the 1:1500 dilution to 27 mL of sterile 5.0% calcium chloride solution.

8. **Test of Reagent:**

Determination of spore preparation LD₅₀ - The guinea pig lethal dose fifty (LD₅₀) was determined by injecting 400- to 500-gram guinea pigs intramuscularly with tenfold dilutions of IRP 313 suspended in 5% calcium chloride solution. The guinea pig LD₅₀ was calculated by the method of Reed and Muench and found to be 10^{6.2091} per 0.5 mL of spore suspension.

Determination of culture purity - IRP 313 was tested for purity and found to be a pure culture of *C. sordellii* based on cellular and colony morphology, biochemical reactions, and analysis of acid and alcohol products.

9. Container Size, Type, Weight, or Volume: Five-mL glass vials containing 1.75 mL of spore suspension.

10. Storage Conditions: Store at $-70^{\circ}\pm 5^{\circ}\text{C}$.

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

12. Origin and Passage History: *C. sordellii* strain 1732, used to produce IRP 313, was obtained from Jensen-Salsbery Laboratories, Inc., on June 3, 1967. The number of passages is unknown.

13. Method of Preparation: *C. sordellii* spores were cultivated in 500-mL Erlenmeyer flasks on the surface of agar medium containing proteose peptone, trypticase, yeast extract, and beef muscle digest. The cultures were incubated at 35°C for 5 days in an anaerobic glove box containing 85% nitrogen (N_2), 10% hydrogen (H_2), and 5% carbon-dioxide (CO_2). The spores were washed from the agar surface with sterile 0.015 M phosphate buffered saline, pH 7.0, 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: CVB@aphis.usda.gov

Reagent orders forms (APHIS 2018) are available from:
https://www.aphis.usda.gov/library/forms/pdf/APHIS_2018.pdf

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