

**United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol**

SAM 200

**Supplemental Assay Method for Potency Testing Products Containing
Clostridium chauvoei Antigen**

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Supplemental Assay Method for Potency Testing Products Containing *Clostridium chauvoei* Antigen

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Supplemental Assay Method for Potency Testing Products Containing *Clostridium chauvoei* Antigen

1. Introduction

This Supplemental Assay Method (SAM) describes procedures for potency testing biological products containing *Clostridium chauvoei* antigen as prescribed in title 9, *Code of Federal Regulations* (9 CFR), section 113.106. For products that require 2 vaccinations, guinea pigs are vaccinated twice, 21 to 23 days apart, and challenged with a standard dose of virulent *C. chauvoei* spores 14 to 15 days following the second vaccination. For products that require a single dose, guinea pigs are vaccinated and challenged with a standard dose of virulent *C. chauvoei* spores 35 to 38 days following vaccination. This is a 2-stage test in which the second stage is applied only when exactly 2 vaccinated guinea pigs die in the first stage.

2. Materials

2.1 Equipment/instrumentation

Equivalent equipment or instrumentation may be substituted for any brand name listed below.

- Mixer, vortex-type

2.2 Reagents/supplies

Equivalent reagents or supplies may be substituted for any brand name listed below.

- 2.2.1** *Clostridium chauvoei* challenge culture, IRP 631, provided by the Center for Veterinary Biologics (CVB).
- 2.2.2** Sodium chloride, reagent grade
- 2.2.3** Calcium chloride dihydrate, reagent grade
- 2.2.4** Syringes, needle-locking, 3-mL, 5-mL, and 10-mL
- 2.2.5** Needles, 23-gauge x 1-inch
- 2.2.6** Pipettes, 2-mL, 5-mL, and 25-mL
- 2.2.7** Glass dilution bottles or flasks, various sizes
- 2.2.8** Glass serum bottles, 50-mL

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2.2.9 Rubber stoppers, 13 x 20-mm, and aluminum caps for serum bottles

2.2.10 Water, deionized or distilled, or water of equivalent purity

2.3 Test animals

Guinea pigs, 300-500 grams. (Eight guinea pigs are required for each serial to be tested. Five additional guinea pigs are required as controls for each 1-4 serials tested. All guinea pigs used for a test series should be from the same source and housed and fed in the same manner.)

3. Preparation for the Test

3.1 Personnel qualifications/training

Technical personnel should have working knowledge of the use of general laboratory chemicals, equipment, and glassware and have specific training and experience in the handling of live bacterial cultures and the handling of laboratory animals.

3.2 Selection and handling of test animals

3.2.1 Select guinea pigs that are healthy and free of external parasites and have an unblemished hair coat.

3.2.2 Guinea pigs of either sex or any color may be used.

3.2.3 Examine guinea pigs the day they are received. House appropriately allowing adequate space. (Do not mix sexes in any 1 cage.)

3.2.4 Instruct the animal caretakers not to clean the cages following challenge.

3.2.5 When the test is concluded, instruct the animal caretakers to euthanize and incinerate the guinea pigs and contaminated bedding and to sanitize the contaminated cages.

3.3 Preparation of supplies

3.3.1 Sterilize all glassware before use.

3.3.2 Make sure all supplies (pipettes, syringes, needles, rubber stoppers, etc.) are sterile.

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3.4 Preparation of reagents

3.4.1 *Clostridium chauvoei* challenge culture

The *Clostridium chauvoei* challenge culture, IRP 631, is suspended in 50% glycerol. Each vial contains approximately 1.5 mL. Store vials of spore suspension lower than -60°C.

3.4.2 Calcium chloride solution, 7.5%

Dissolve 27 grams of calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) in 360 mL of distilled water. Autoclave at $\geq 121^\circ\text{C}$ for 25-30 minutes following manufacturer's recommendations. Cool solution on ice and aseptically dispense 27 mL into a 50-mL serum bottle.

Note: Prepare the Calcium chloride solution on the day of the challenge. Keep the solution on ice until used.

3.4.3 Sodium chloride solution, 0.85%

Dissolve 1.7 grams of Sodium Chloride (NaCl) in 200 mL of distilled water. Autoclave at $\geq 121^\circ\text{C}$ for 25-30 minutes following manufacturer's recommendations. Cool solution on ice and dispense aseptically 99 mL into a 160-mL glass dilution bottle or 125-mL flask and a 36 mL quantity into a 50-mL glass serum bottle.

Note: Prepare the Sodium chloride solution on the day of the challenge. Keep the solution on ice while making dilutions.

4. Performance of the Test

4.1 Vaccination of test animals

4.1.1 Check the label on each container of product to be tested for identity and recommended field dose. The guinea pig dose is 1/5 of the recommended calf dose.

4.1.2 Thoroughly mix the product by striking the container against the palm of the hand at least 25 times before the syringes are filled. Use 5-mL or 10-mL syringes, fitted with 23-gauge x 1-inch needles.

4.1.3 Vaccinate 8 guinea pigs with each serial of product. Administer the vaccine subcutaneously in the ventral thoracic area. Administer the first vaccination on the RIGHT side of the guinea pig.

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4.1.4 For 2-dose products, administer the second vaccination 21 to 23 days after the first. Administer the second vaccination subcutaneously in the ventral thoracic area on the LEFT side of the guinea pig.

4.2 Timing of challenge

4.2.1 Challenge 8 guinea pigs per serial of product 14 to 15 days after the second vaccination for 2-dose products or 35 to 38 days after vaccination for single-dose products.

4.2.2 Challenge the nonvaccinated control guinea pigs at the same time. The 5 nonvaccinated guinea pigs may serve as controls for up to 4 serials of product.

4.3 Preparation and administration of challenge

4.3.1 Thaw a vial of culture suspension at room temperature and use immediately.

4.3.2 Mix the contents thoroughly by shaking the vial vigorously.

4.3.3 Use a 2-mL pipette to remove 1.2 mL of spore suspension from the vial. Transfer 1 mL of spore suspension into a glass dilution bottle or flask containing 99 mL of cold, sterile 0.85% NaCl solution (1:100 dilution).

Note: The *C. chauvoei* spore suspension is viscid and must be pipetted with care to ensure delivery of the complete volume.

4.3.4 Use a 2-mL pipette to remove 1.2 mL of well-mixed 1:100 dilution of spore suspension. Transfer 1.0 mL of the diluted spore suspension into a glass dilution bottle containing 36 mL of cold, sterile 0.85% NaCl solution (1:3700 dilution).

4.3.5 Use a 5-mL pipette to remove 5.0 mL of well-mixed 1:3700 dilution of spore suspension. Transfer 3 mL into a 50-mL serum vial containing 27 mL of cold, sterile calcium chloride solution (final concentration of calcium chloride solution equals approximately 5%). Cap and seal the bottle. Mix this 1:37,000 challenge suspension thoroughly and place it on ice. Allow the challenge suspension to remain on ice approximately 10 minutes before inoculating the first guinea pig.

4.3.6 Mix the challenge thoroughly before each syringe is filled. Use 3-mL needle-locking syringes fitted with 23-gauge x 1-inch needles to inoculate the guinea pigs. Inoculate each guinea pig intramuscularly in the thigh of the right or left hind leg with 0.5 mL of challenge material. The challenge dilution contains approximately 100 guinea pig lethal dose 50 (LD₅₀) per 0.5 mL.

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Note: Each 30-mL volume of the 1:37,000 challenge dilution may be used to challenge up to 40 vaccinates and 5 nonvaccinated controls. This constitutes a challenge unit. Additional 30-mL volumes of challenge must be prepared if additional challenge units are required.

4.3.7 Complete the inoculation of the guinea pigs in each challenge unit within approximately 30 minutes from the time the challenge dilution is prepared.

4.4 Observation of guinea pigs following challenge

The guinea pigs are observed for 72 hours following challenge. Deaths are recorded. The test animals are also examined for sores, edema, and general physical condition. These observations are also noted on the daily record. The test is concluded at the end of the 72 hours.

Note: Moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power may be humanely euthanized and considered as deaths as outlined in 9 CFR 117.4.

5. Interpretation of the Test Results

The test is interpreted as prescribed in 9 CFR 113.106. For a valid test, at least 80 percent of the controls shall die within the 3-day postchallenge observation period. If this requirement is met, the results of the potency test shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of deaths for a satisfactory test	Cumulative total number of deaths for an unsatisfactory test
1	8	8	1 or less	3 or more
2	8	16	4 or less	5 or more

The second stage is required only when exactly 2 animals die in the first stage. The second stage test is performed in a manner identical to the first stage test.

6. Report of Test Results

Report results of the test(s) as described by standard operating procedures.

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7. References

7.1 Title 9, *Code of Federal Regulations*, section 113.106, U.S. Government Printing Office, Washington, DC.

7.2 History of *Clostridium chauvoei* IRP 631: This spore suspension was prepared October 16, 2015. It replaces IRP 509 (04) and was prepared from *C. chauvoei* IRP 206, which was prepared from *C. chauvoei* Lot F, obtained from American Cyanamid in 1962.

8. Summary of Revisions

Version .08

- The coversheet, including contact information, has been updated.
- Minor formatting changes and procedure clarification to match current practices.

Version .07

- Update 3.4.3 to reflect correct sodium chloride dispense volumes.

Version .06

- Challenge reagent lot information updated.

Version .05

- Bacteriology Section Leader updated.
- Minor word changes for clarification of procedures.

Version .04

- The Contact information has been updated.

Version .03

- The document number has been changed from BBSAM0200 to SAM 200.

Version .02

This document was revised to clarify the practices currently in use at the Center for Veterinary Biologics and to provide additional detail. While no significant changes were made that impact the outcome of the test, the following changes were made to the document:

- 2.2.7 16 x 125-mm screw-capped test tubes have been changed to 160-ml glass dilution bottles.
- 2.3.1 The acceptable guinea pig weight has been changed to match the regulation (9 CFR 113.106).
- 4.4.2 The humane endpoint wording has been slightly revised for consistency.
- IRP 434 has been changed to IRP 509(04) throughout the document.
- The contact person has been changed to Janet M. Wilson.