United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol

SAM 209

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

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1. Introduction

This Supplemental Assay Method (SAM) describes procedures for potency testing biological products containing *Clostridium haemolyticum* antigen as prescribed in title 9, *Code of Federal Regulations* (9 CFR), section 113.107. 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. haemolyticum* 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. haemolyticum* 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 unless specified as required for the test.

2.2.1 *C. haemolyticum* challenge culture, IRP 526 (06) (This culture must be obtained from the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Center for Veterinary Biologics (CVB), Ames, IA 50010.)

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, 10-mL, and 25-mL

2.2.7 Glass screw-cap tubes, 16 x 125-mm

2.2.8 Polypropylene screw-top conical tubes, 50-mL

2.2.9 Glass serum bottles, 50-mL

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

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

2.3 Test animals

Guinea pigs, 300-500 g. (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 must 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 need a working knowledge of the use of general laboratory chemicals, equipment, and glassware. In addition, personnel need specific training and experience in the handling of live bacterial cultures and the handling of guinea pigs.

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 disposable supplies (pipettes, syringes, needles, rubber stoppers, etc.) are sterile.

3.4 Preparation of reagents

3.4.1 *C. haemolyticum* challenge culture

The challenge culture, *C. haemolyticum* IRP 526 (06), is suspended in 50% glycerol. Each vial contains approximately 0.8 mL. Store vials of spore suspension at -50° to -90°C.

3.4.2 Calcium chloride solution

Dissolve 13.5 g of calcium chloride dihydrate ($CaCl_2$-$2H_2O$) in 180 mL of distilled water. Autoclave at ≥121°C for 25-30 minutes following manufacturer's recommendations. Cool and aseptically dispense 27 mL into a 50-mL serum bottle.

Note: Prepare the $CaCl_2$ solution on the day of the challenge. Keep the solution on ice until used.

3.4.3 NaCl solution, 0.85%

Dissolve 1.7 g of NaCl in 200 mL of distilled water. Autoclave at ≥121°C for 25-30 minutes following manufacturer's recommendations. Cool and dispense aseptically 4.5-mL and 9-mL quantities into 16 x 125-mm tubes.

Note: Prepare the NaCl 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.

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 of challenge

4.3.1 Thaw a vial of challenge culture, IRP 526 (06), 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 0.7 mL of spore suspension from the vial. Pipette 0.5 mL of spore suspension into a glass tube containing 4.5 mL of cold, sterile 0.85% NaCl solution (1:10 dilution). Further dilute the spore suspension by adding 1.0 mL of well mixed 1:10 dilution to 9.0 mL of cold, sterile 0.85% NaCl solution (1:100 dilution).

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

4.3.4 Use a 2-mL pipette to remove 2 mL of well-mixed spore suspension diluted 1:100. Transfer 1 mL into a 50-mL serum vial containing 39 mL of cold, sterile calcium chloride solution (final concentration of calcium chloride solution equals approximately 5%). Cap and seal the bottle. Mix this 1:4000 challenge suspension thoroughly and place on ice. Allow the challenge suspension to remain on ice approximately 10 minutes before inoculating the first guinea pig.

4.3.5 Mix the challenge thoroughly before each syringe is filled. Use 3-mL 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 (LD_{50}) per 0.5 mL.

Note: Each 40-mL volume of the 1:4000 challenge dilution may be used to challenge up to 40 vaccinates and 5 nonvaccinated controls. This constitutes a challenge unit. Additional 40-mL volumes of challenge must be prepared if additional challenge units are required.

4.3.6 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

Observe the guinea pigs for 72 hours following challenge and record deaths. Also examine the test animals for sores, edema, and general physical condition. Record these observations 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 Test Results

The test is interpreted as prescribed in 9 CFR 113.107. For a valid test, at least 80% 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.

7. References

7.1 Title 9, *Code of Federal Regulations*, section 113.107, U.S. Government Printing Office, Washington, DC.

7.2 History of reagents: *C. haemolyticum*, IRP 89 (strain 7170), used to prepare IRP 526 (06), was obtained from Montana State University, Bozeman, Montana, in 1962. The number of passages is unknown.

8. Summary of Revisions

Version .06

- Updated **3.4.3** to reflect correct sodium chloride dispense volumes.
- Updated **7.2** to correct lot number.
- The Director was updated on the cover page.

Version .05

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

Version .04

- The Contact information has been updated.
- The IRP number has been updated throughout the document from 477 to 526 (06).

Version .03

- The document number has been changed from BBSAM0209 to SAM 209.

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:

- IRP 315 has been changed to IRP 477 throughout the document.

- The acceptable guinea pig weight has been changed to match the regulation (9 CFR 113.107).

- The humane endpoint wording has been slightly revised for consistency.

- The contact person has been changed to Janet M. Wilson.