United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol

SAM 213

Supplemental Assay Method for Potency Testing of *Clostridium botulinum* Type C Bacterin-Toxoids

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Contact:	Email: Methodsrequest.notification@usda.gov Phone: Center for Veterinary Biologics, 515-337-6100

United States Department of Agriculture
Animal and Plant Health Inspection Service
P. O. Box 844
Ames, IA 50010

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1. Introduction

This Supplemental Assay Method (SAM) describes procedures for potency testing biological products containing *Clostridium botulinum* Type C antigen as prescribed in title 9, *Code of Federal Regulations* (9 CFR), part 113.110. Mink are vaccinated subcutaneously then challenged intraperitoneally 21 to 28 days later with a standard dose of *C. botulinum* Type C toxin.

2. Materials

2.1 Equipment/instrumentation

2.1.1 Vortex-type mixer

2.1.2 Refrigerator, 2°- 7°C

2.1.3 Freezer, -70°C or lower

2.2 Reagents/supplies

2.2.1 *C. botulinum* Type C toxin, current lot supplied by CVB

2.2.2 Pipettes, 1-mL, 2-mL, 5-mL, 10-mL, and 25-mL

2.2.3 Syringes, needle-locking, 3-mL, and 5-mL

2.2.4 Needles, 23-gauge x 1-inch

2.2.5 Serum vials, 50-mL, with caps and seals

2.2.6 M/15 phosphate buffered physiological saline with 0.2% gelatin

2.3 Test animals

2.3.1 Mink, young adult, from the same source and similar age. Vaccinate a group of 5 mink for each product tested. Three additional mink are held as unvaccinated controls.

2.3.2 Mice, 16-20 g, fifty mice are required to determine the LD_{50} of the challenge inoculum. All mice must be from the same source colony.

3. Preparation for the Test

3.1 Personnel qualifications/training

Technical personnel need to have a working knowledge of the use of general laboratory chemicals, equipment, and glassware; and have specific training and experience in the safe handling of clostridial toxins. Personnel need specific training in the care and handling of laboratory animals.

3.2 Selection and handling of test animals

3.2.1 Healthy, young adult mink are caged individually for the potency test.

3.2.2 Mink of either sex or any color may be selected.

3.2.3 Susceptible mink are required. They must not have been vaccinated with any *C. botulinum* Type C-containing product.

3.3 Preparation of supplies

3.3.1 Sterilize all glassware before use.

3.3.2 Use only sterile supplies (pipettes, syringes, needles, etc.).

3.3.3 Operate all equipment according to the manufacturers' instructions.

3.4 Preparation of reagents

3.4.1 M/15 phosphate buffered saline with 0.2% gelatin

NaCl	8.5 g
Gelatin	2.0 g
M/15 phosphate buffer at pH 7.4	1000.0 mL

Stock solution of M/15 KH_2PO_4 (Solution A):

Dissolve 9.072 grams of KH_2PO_4 in one liter of distilled water.

Stock solution of M/15 Na_2HPO_4 (Solution B):

Dissolve 9.465 grams of Na_2HPO_4 in one liter of distilled water.

One hundred ninety-two (192) milliliters of Solution A are mixed with 808 mL of Solution B to provide 1000 mL of M/15 phosphate buffer at pH 7.4.

Eight and one-half (8.5) grams of NaCl and 2 grams of gelatin are dissolved in the 1000 mL of M/15 phosphate buffer (pH 7.4). It is then autoclaved at ≥ 121°C for 30 minutes. Store at 2°- 7°C for up to 6 months.

3.4.2 *C. botulinum* Type C toxin

C. botulinum Type C challenge toxin, supplied by the Center for Veterinary Biologics (CVB). Store and use according to the reagent data sheet.

4. Performance of the Test

4.1 Vaccination

Vaccinate the mink subcutaneously with the mink dose recommended on the label of the test serial final container using 5-mL sterile, disposable syringes with 23-gauge x 1-inch disposable needles.

4.2 Challenge of immunity

4.2.1 Challenge both vaccinated mink and unvaccinated control mink 21 to 28 days after the vaccination.

4.2.2 Dilute *C. botulinum* Type C toxin according to the reagent data sheet.

4.2.3 Inoculate the mink intraperitoneally with 0.5 mL of challenge preparation using a 3-mL disposable syringe and 23-gauge x 1-inch needle.

4.2.4 Immediately following the mink inoculations, prepare the additional toxin dilutions of 1:100,000, 1:200,000, 1:400,000, and 1:800,000 in M/15 phosphate buffered physiological saline with 0.2% gelatin (see **Appendix**). Inoculate intraperitoneally each of 10 mice with 0.5 mL of each final toxin dilution. In most tests, at least 9 of 10 mice inoculated with the 1:100,000 dilution will die within 7 days and at least 9 of 10 mice inoculated with the 1:800,000 dilution will survive for 7 days.

5. Interpretation of the Test Results

Observe the mink for 7 days after challenge for signs of botulism and note deaths. For a valid test, the unvaccinated controls must die. If the test is valid and at least 80% of the vaccinated mink do not remain free of botulism, the serial is unsatisfactory.

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

6. Report of Test Results

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

7. References

Title 9, *Code of Federal Regulations,* part 113.110, U.S. Government Printing Office, Washington, DC.

8. Summary of Revisions

Version .04

- Updated coversheet and contact information.
- Removed challenge lot identification and corresponding storage and use information.

Version .03

- Section Leader and Director information have been updated.
- Toxin lot number and corresponding use dilutions have been updated throughout the document.

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:

- The document number has been changed from BBSAM0213 to SAM 213.

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- The Standard Requirement has been changed from 9 CFR 113.95 to 9 CFR 113.110.
- The Contact information has been updated.

- **Section 4.3** information has been moved to **Section 5.**
- Toxin lot numbers and corresponding use dilutions have been updated throughout the document.

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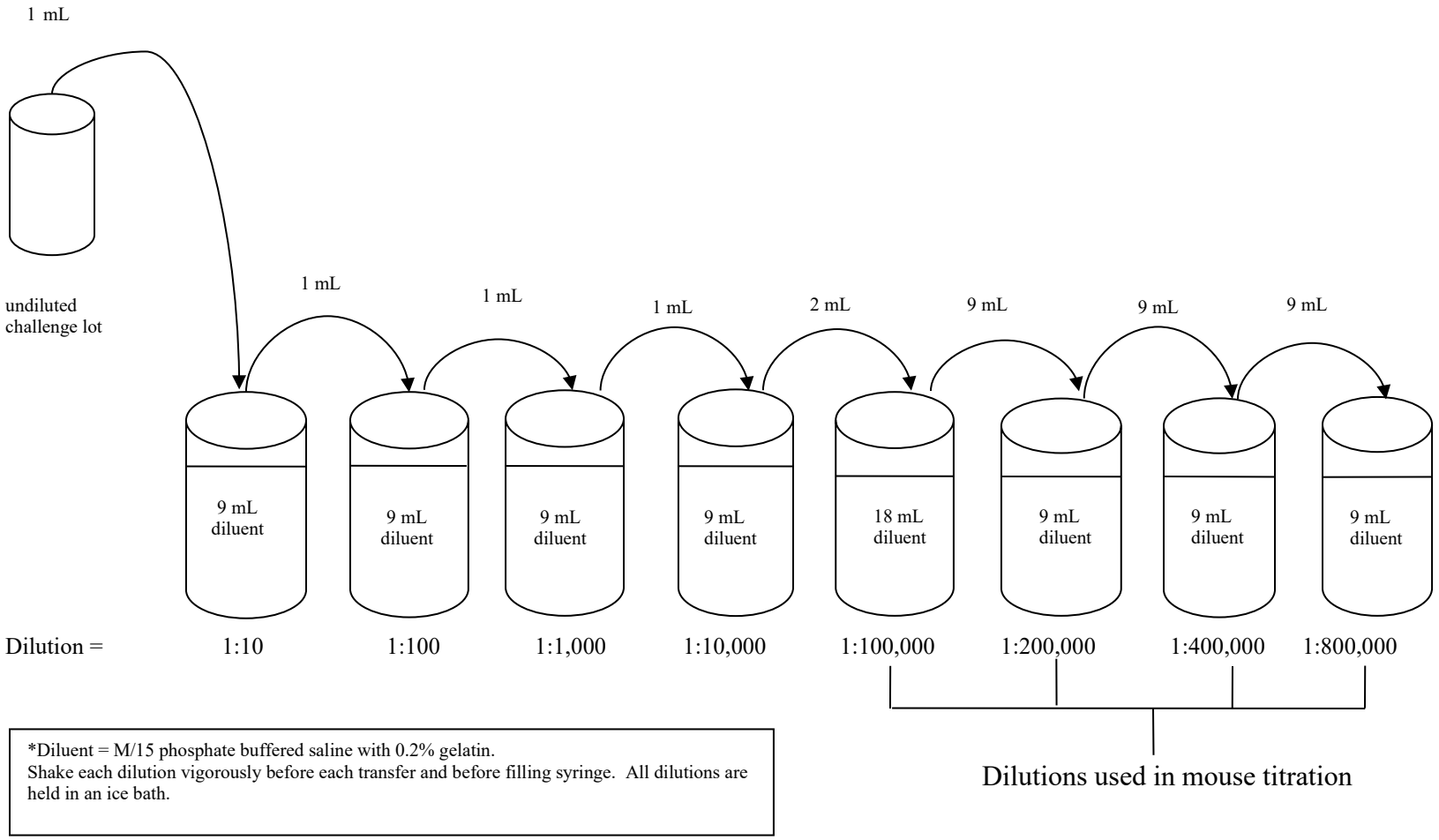
Appendix: Potential Mink Challenge and LD_{50} Dilution Preparations



*Diluent = M/15 phosphate buffered saline with 0.2% gelatin.
Shake each dilution vigorously before each transfer and before filling syringe. All dilutions are held in an ice bath.