

United States Department of Agriculture  
Center for Veterinary Biologics  
Testing Protocol

SAM 202

Supplemental Assay Method for Potency Testing *Clostridium perfringens*  
Type C Beta Antitoxins

Date: December 12, 2017

Number: SAM 202.06

Supersedes: SAM 202.05, June 18, 2014

Standard Requirement: 9 CFR 113.454

Contact: Janet M. Wilson, (515) 337-7245

Approvals: /s/Larry R. Ludemann Date: 19Dec17  
Larry R. Ludemann, Section Leader  
Bacteriology

/s/Paul J. Hauer Date: 27Dec17  
Paul J. Hauer, Director  
Policy, Evaluation, and Licensing  
Center for Veterinary Biologics

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

Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by USDA and does not imply its approval to the exclusion of other products that may be suitable.

Entered into CVB Quality Management System by: <u>/s/Linda S. Snavely</u> <u>27Dec17</u> Linda S. Snavely Date Quality Management Program Assistant
--

**UNCONTROLLED COPY**

Supplemental Assay Method for Potency Testing *Clostridium perfringens* Type C Beta Antitoxins

**Table of Contents**

- 1. Introduction**
- 2. Materials**
  - 2.1 Equipment/instrumentation**
  - 2.2 Reagents/supplies**
  - 2.3 Test animals**
- 3. Preparation for the Test**
  - 3.1 Personnel qualifications/training**
  - 3.2 Preparation of equipment/instrumentation**
  - 3.3 Preparation of reagents/control procedures**
- 4. Performance of the Test**
  - 4.1 Toxin neutralization**
  - 4.2 Inoculation of mice**
- 5. Interpretation of the Test Results**
  - 5.1 Criteria for a valid test**
  - 5.2 Interpretation of serial results**
- 6. Report of Test Results**
- 7. References**
- 8. Summary of Revisions**

Supplemental Assay Method for Potency Testing *Clostridium perfringens* Type C Beta Antitoxins

## 1. Introduction

This Supplemental Assay Method (SAM) describes the method used to determine the beta antitoxin content of *Clostridium perfringens* type C antitoxins as prescribed by the title 9, *Code of Federal Regulations* (9 CFR), part 113.454. The antitoxin is titrated by a toxin-antitoxin neutralization test using mice as an indicator.

## 2. Materials

### 2.1 Equipment/instrumentation

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

- 2.1.1 Mixer, vortex type
- 2.1.2 Freezer, -70°C
- 2.1.3 Micropipettes, 100-µL and 1000-µL
- 2.1.4 Refrigerator, 2°- 7°C
- 2.1.5 Autoclave

### 2.2 Reagents/supplies

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

- 2.2.1 *C. perfringens* type C (beta) standard antitoxin IRP 637 (available from the Center for Veterinary Biologics (CVB))
- 2.2.2 *C. perfringens* type C (beta) standard toxin IRP 624 (available from the CVB)
- 2.2.3 Peptone diluent
- 2.2.4 Tubes, various sizes with lids
- 2.2.5 Pipettes, various sizes
- 2.2.6 Syringes, 1-cc, luer-lock

**Supplemental Assay Method for Potency Testing *Clostridium perfringens* Type C Beta Antitoxins**

**2.2.7** Needles, 25- to 27-gauge x 1- to 1 1/4-inch

**2.2.8** Screw-top Erlenmeyer flask, 500-mL, with cap

**2.2.9** Water, distilled or deionized, or water of equivalent purity

**2.2.10** Tips for micropipettes

**2.3 Test animals**

White Swiss nonpregnant female mice, 16-20 g (Five mice are required for each toxin-antitoxin mixture.)

**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 and must have specific training and experience in the safe handling of clostridial toxins. Personnel need specific training in the care and handling of laboratory mice.

**3.2 Preparation of equipment/instrumentation**

All equipment is operated according to manufacturers' instructions.

**3.3 Preparation of reagents/control procedures**

**3.3.1 Peptone diluent**

Peptone (Difco)	8 g
NaCl, reagent grade	2 g
Water q.s. to	800 mL

Dissolve peptone and sodium chloride in water. Adjust pH to 7.2 with 1N sodium hydroxide.

Dispense and autoclave with caps loosened at  $\geq 121^{\circ}\text{C}$  for 25 to 30 minutes following manufacturer's recommendations. Cool flasks and tighten caps. Store diluent at  $2^{\circ}$ -  $7^{\circ}\text{C}$  for up to 3 months.

Supplemental Assay Method for Potency Testing *Clostridium perfringens* Type C Beta Antitoxins

**3.3.2** Preparation of *C. perfringens* type C standard beta antitoxin

1. *Clostridium perfringens* type C antitoxin IRP 637 contains 550 units of beta antitoxin per mL (AU/mL) and has been standardized against the World Health Organization *C. perfringens* (*C. welchii*) type C International antitoxin. Each vial contains 0.6 mL of antitoxin.
2. A dilution of standard beta antitoxin containing 10 (AU/mL) is used in the toxin-neutralization test. Follow the current Reagent Data Sheet to prepare the dilution.

**3.3.3** Preparation of *C. perfringens* type C standard beta toxin

1. Follow the current Reagent Data Sheet to prepare the dilution of *C. perfringens* type C beta toxin IRP 624. IRP 624, diluted 1:10, is stable when stored at  $-70^{\circ}\pm 10^{\circ}\text{C}$ .

**Note: A volume of 0.5 mL of standard beta toxin and 0.5 mL of peptone diluent represents 10 L<sub>o</sub> doses. A volume of 0.9 mL of the standard beta toxin and 0.1 mL of peptone diluent represents 10 L<sub>+</sub> doses (see Sections 4.1.1 and 4.1.2). For the purposes of this SAM, 10 L<sub>o</sub> dose is defined as the greatest amount of toxin that, when mixed with 10 AU, results in 100% survival of all mice inoculated intravenously (IV) with 0.2 mL of this mixture. The 10 L<sub>+</sub> dose is defined as the least amount of toxin that, when mixed with 10 AU, results in the death of 80%-100% of all mice inoculated IV with 0.2 mL of this mixture.**

**4. Performance of the Test**

**4.1 Toxin neutralization**

**4.1.1** Product and standard beta toxin

1. Mix a sufficient volume of standard beta toxin and peptone diluent (0.5 mL of standard beta toxin and 0.5 mL peptone diluent (10 L<sub>o</sub> doses)) for each product antitoxin dilution and the L<sub>o</sub> control using a 17 x 120-mm conical tube.

**Supplemental Assay Method for Potency Testing *Clostridium perfringens* Type C Beta Antitoxins**

2. Add 1 mL of each product antitoxin dilution (see table below) to 1 mL of the standard beta toxin-peptone diluent mixture (10 L<sub>o</sub> doses) in 17 x 100-mm snap-top tubes. Mix each tube with a vortex-type mixer.

Int'l AU tested	Unknown Antitoxin	10 L <sub>o</sub> doses	
		Std Toxin	Diluent
500	1 mL diluted 1:50 (0.5 mL product + 24.5 mL dil.)	0.5 mL	0.5 mL
600	1 mL diluted 1:60 (0.5 mL product + 29.5 mL dil.)	0.5 mL	0.5 mL
1200	1 mL diluted 1:120 (0.3 mL product + 35.7 mL dil.)	0.5 mL	0.5 mL

3. Let the mixtures sit at 22°- 26°C (room temperature) for 1 hour.

4. Place tubes in ice.

**4.1.2 Standard beta toxin and standard beta antitoxin controls**

1. Add 1.0 mL of standard beta antitoxin containing 10 AU/mL to 1.0 mL of the standard beta toxin-peptone diluent mixture (10 L<sub>o</sub> doses) in a 17 x 100-mm snap-top tube. Mix well with a vortex-type mixer.
2. Add 1.0 mL of standard beta antitoxin containing 10 AU/mL to 0.9 mL of standard beta toxin and 0.1 mL of peptone diluent (10 L<sub>+</sub> doses) in a 17 x 100-mm snap-top tube. Mix well with a vortex-type mixer.

3. Let the mixtures stand at 22°- 26°C for 1 hour.

4. Place tubes in ice.

**4.2 Inoculation of mice**

**4.2.1** Inject 0.2 mL of each standard beta toxin-product antitoxin mixture into each of 5 mice.

**4.2.2** Inject 0.2 mL of each standard beta toxin-standard beta antitoxin mixture into each of 5 mice.

**4.2.3** Inoculate all mice intravenously into a lateral tail vein. Use 1-cc needle locking syringes fitted with 25- to 27-gauge x 1- to 1 1/4-inch needles.

**4.2.4** Always inoculate the mice receiving the standard beta toxin-standard beta antitoxin mixtures (controls) **last**.

Supplemental Assay Method for Potency Testing *Clostridium perfringens* Type C Beta Antitoxins

4.2.5 Mouse inoculations should be completed within 1 hour of placing the toxin-antitoxin mixtures in the ice.

4.2.6 The test is concluded 24 hours after the mice are inoculated.

**5. Interpretation of the Test Results**

**5.1 Criteria for a valid test**

5.1.1 All 5 mice inoculated with the standard 10 L<sub>o</sub>/10 AU control mixture must survive.

5.1.2 At least 4 of the 5 mice inoculated with the standard 10 L<sub>+</sub>/10 AU control mixture must die.

**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.**

**5.2 Interpretation of serial results**

5.2.1 The product contains at least 500 International Units of beta antitoxin per mL if 5 of the 5 mice inoculated with the 1:50 dilution of product-standard beta toxin mixture survive.

5.2.2 The product contains at least 600 International Units of beta antitoxin per mL if 5 of the 5 mice inoculated with the 1:60 dilution of product-standard beta toxin survive.

5.2.3 The product is considered unsatisfactory if it contains less than 500 International Units of beta antitoxin per mL. (If any mice inoculated with the 1:50 dilution and 10 L<sub>o</sub> doses of standard beta toxin die, the product is considered to contain less than 500 International Units per mL.)

**6. Reporting of Test Results**

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

Supplemental Assay Method for Potency Testing *Clostridium perfringens* Type C Beta Antitoxins

**7. References**

**7.1** Title 9, *Code of Federal Regulations*, part 113.454, U.S. Government Printing Office, Washington, DC.

**7.2** History of toxin: *C. perfringens* type C culture #4414, used to produce IRP 624, and was obtained from Coopers Animal Health, Inc., 1201 Douglas Avenue, Kansas City, Kansas, on July 28, 1975. The number of passages is unknown.

**7.3** History of antitoxin: *C. perfringens* type C (beta) antitoxin IRP 637 was produced in goats hyperimmunized with multiple injections of purified *C. perfringens* type C toxoid and toxin during a 6 month period.

**8. Summary of Revisions**

**Version .06**

- Antitoxin lot IRP 637 replaces IRP 585-A throughout the document.
- Toxin lot IRP 624 replaces IRP 513 (04) throughout the document.
- 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.
- Standard beta antitoxin lot IRP 585 has replaced IRP 486 throughout the document. Due to change in the reagent lot, the use dilution has also changed.

**Version .03**

- The document number has been changed from BBSAM0202 to SAM 202.
- **3.3.3:** The standard beta toxin use dilution has been adjusted.



Supplemental Assay Method for Potency Testing *Clostridium perfringens* Type C Beta Antitoxins

**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 119 has changed to IRP 486 throughout the document.
- IRP 418 has changed to IRP 513(04) throughout the document.
- **4.1** The format and content have been modified to clarify the L<sub>0</sub> and L<sub>+</sub> levels of the Toxin Neutralization process.
- Humane endpoint language has been added.
- Dilution/holding vessel sizes have been added for clarification.
- The contact person has been changed to Janet M. Wilson