(5) Test Interpretation shall be as follows:

(i) If any mice inoculated with the mixture of 1.0 International Unit of Standard Antitoxin and 1.0 Lo doses of Standard Toxin die, the results of the serum neutralization test are inconclusive and shall be repeated: Provided, That, if the test is not repeated, the serial shall be declared unsatisfactory.

(ii) If less than 80 percent of the mice inoculated with the mixture of 1.0 International Unit of Standard Antitoxin and 1.0 L+ doses of Standard Toxin die, the results of the serum neutralization test are inconclusive and shall be repeated: Provided, That, if the test is not repeated, the serial shall be declared unsatisfactory.

(iii) If any mice inoculated with the mixture of 1.0 ml undiluted serum with 1.0 Lo dose of Standard Toxin die, the serum is considered to contain less than 1.0 International Units per ml.

(iv) If the single pooled serum from seven or more rabbits contains less than 1.0 International Unit per ml, the serial is unsatisfactory.

(§ 113.110 Clostridium Botulinum Type C Bacterin-Toxoid.)

Clostridium Botulinum Type C Bacterin-Toxoid shall be produced from a culture of Clostridium botulinum Type C which has been inactivated and is nontoxic. Each serial of biological product containing Clostridium botulinum Type C fraction shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) Purity test. Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) Safety test. Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.33(b).

(c) Potency test. Bulk or final container samples of completed product from each serial shall be tested for potency using susceptible mink as test animals. At least five vaccinates and three unvaccinated controls of the same source and approximately the same age shall be used.

(1) Each of the vaccinates shall be injected subcutaneously with the dose recommended on the label for mink. Twenty-one to twenty-eight days post-injection, the vaccinates and the controls shall be challenged intraperitoneally with botulinum Type C toxin which has been titrated in mice to provide for a 10^4.0 mouse MLD dose. The titration technique shall include inoculation of the mice intraperitoneally.

(2) The vaccinates and controls shall be observed for 7 days post-challenge and signs of botulism and deaths noted. For a valid test, the controls shall die of botulism. If the test is valid and 80 percent of the vaccinates do not remain free of botulism, the serial is unsatisfactory.

(§ 113.111 Clostridium Perfringens Type C Toxoid and Bacterin-Toxoid.)

Clostridium Perfringens Type C Toxoid and Clostridium Perfringens Type C Bacterin-Toxoid shall be produced from a culture of Clostridium perfringens Type C which has been inactivated and is nontoxic. Each serial shall meet the applicable requirements in § 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) Purity test. Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) Safety test. Bulk or final container samples of completed product from each serial shall be tested for safety as provided in § 113.33(b).

(c) Potency test. Bulk or final container samples of completed product from each serial shall be tested for potency using the Beta toxin-neutralization test provided in this paragraph.
§ 113.111

(1) When used in this test, the following words and terms shall mean:
   (i) **International antitoxin unit.** (I.U.) That quantity of Beta Antitoxin which reacts with \( L_0 \) and \( L^{\partial} \) doses of Standard Toxin according to their definitions.
   (ii) \( L_0 \) dose. The largest quantity of toxin which can be mixed with one unit of Standard Antitoxin and not cause sickness or death in injected mice.
   (iii) \( L^{\partial} \) dose. The smallest quantity of toxin which can be mixed with one unit of Standard Antitoxin and cause death in at least 80 percent of injected mice.
   (iv) **Standard antitoxin.** The Beta Antitoxin preparation which has been standardized as to antitoxin unitage on the basis of the International Clostridium perfringens Beta Antitoxin Standard and which is either supplied by or acceptable to Animal and Plant Health Inspection Service. The antitoxin unit value shall be stated on the label.
   (v) **Standard toxin.** The Beta toxin preparation which is supplied by or is acceptable to Animal and Plant Health Inspection Service.
   (vi) **Diluent.** The solution used to make proper dilutions prescribed in this test. Such solutions shall be made by dissolving 1 gram of peptone and 0.25 grams of sodium chloride in each 100 ml of distilled water; adjusting the pH to 7.2; autoclaving at 250 °F for 25 minutes; and storing at 4 °C until used.

(2) Each of at least eight rabbits of a strain acceptable to APHIS, each weighing 4–8 pounds, shall be injected subcutaneously with not more than half of the largest recommended dose for any species indicated on the product label. A second equivalent dose shall be given not less than 20 days nor more than 23 days after the first does.

(3) Each of at least eight rabbits of a strain acceptable to APHIS, each weighing 4–8 pounds, shall be injected subcutaneously with not more than half of the largest recommended dose for any species indicated on the product label. A second equivalent dose shall be given not less than 20 days nor more than 23 days after the first does.

(4) The antitoxin content of the rabbit sera shall be determined as follows:
   (i) Make a dilution of Standard Antitoxin to contain 10 International Units of antitoxin per ml.
   (ii) Make one dilution of Standard Toxin to contain \( 10 \ L_0 \) doses per ml and make a second dilution of Standard Toxin to contain \( 10 \ L^{\partial} \) doses per ml.
   (iii) Combine 10 International Units of Standard Antitoxin with \( 10 \ L_0 \) doses of diluted Standard Toxin and combine 10 International Units of Standard Antitoxin with \( 10 \ L^{\partial} \) doses of diluted Standard Toxin.
   (iv) Combine 1 ml of undiluted serum with \( 10 \ L_0 \) doses of diluted Standard Toxin.
   (v) Neutralize all toxin-antitoxin mixtures at room temperature for 1 hour and hold in ice water until injections of mice can be made.

(5) Test Interpretation shall be as follows:
   (i) If any mice inoculated with the mixture of 10 International Units of Standard Antitoxin and \( 10 \ L_0 \) doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: Provided, That, if the test is not repeated, the serial shall be declared unsatisfactory.
   (ii) If less than 80 percent of the mice inoculated with mixture of 10 International Units of Standard Antitoxin and \( 10 \ L^{\partial} \) doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: Provided, That, if the test is not repeated, the serial shall be declared unsatisfactory.
   (iii) If any mice inoculated with the mixture of serum with \( 10 \ L_0 \) doses of
§ 113.112 Clostridium Perfringens Type D Toxoid and Bacterin-Toxoid.

Clostridium Perfringens Type D Toxoid and Clostridium Perfringens Type D Bacterin-Toxoid shall be produced from a culture of Clostridium perfringens Type D which has been inactivated and is nontoxic. Each serial shall meet the applicable requirements in §113.100 and shall be tested for purity, safety, and potency as prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) Purity test. Final container samples of completed product from each serial and each subserial shall be tested for viable bacteria and fungi as provided in §113.26.

(b) Safety test. Bulk or final container samples of completed product from each serial shall be tested for safety as provided in §113.33(b).

(c) Potency test. Bulk or final container samples of completed product from each serial shall be tested for potency using the Epsilon toxin-neutralization test provided in this paragraph.

(i) When used in this test, the following words and terms shall mean:

(I) International antitoxin unit. (I.U.) That quantity of Epsilon Antitoxin which reacts with $L_0$ and $L_\partial$ doses of Standard Toxin according to their definitions.

(ii) $L_0$ dose. The largest quantity of toxin which can be mixed with one-tenth unit of Standard Antitoxin and not cause sickness or death in injected mice.

(iii) $L_\partial$ dose. The smallest quantity of toxin which can be mixed with one-tenth unit of Standard Antitoxin and cause death in at least 80 percent of injected mice.

(iv) Standard antitoxin. The Epsilon Antitoxin preparation which has been standardized as to antitoxin unitage on the basis of the International Clostridium perfringens Epsilon Antitoxin Standard and which is either supplied by or acceptable to Animal and Plant Health Inspection Service. The antitoxin unit value shall be stated on the label.

(v) Standard toxin. The Epsilon toxin preparation which is supplied by or is acceptable to Animal and Plant Health Inspection Service.

(vi) Diluent. The solution used to make proper dilutions prescribed in this test. Such solutions shall be made by dissolving 1 gram of peptone and 0.25 gram of sodium chloride in each 100 ml of distilled water; adjusting the pH to 7.2; autoclaving at 250 °F for 25 minutes; and storing at 4 °C until used.

(2) Each of at least eight rabbits of a strain acceptable to APHIS, each weighing 4-8 pounds, shall be injected subcutaneously with not more than half of the largest recommended dose for any species indicated on the product label. A second equivalent dose shall be given not less than 20 days nor more than 23 days after the first dose.

(3) Fourteen to seventeen days after the second dose, all surviving rabbits shall be bled, and the serum tested for antitoxin content.

(i) At least seven rabbits are required to make an acceptable serum pool.

(ii) Equal quantities of serum from each rabbit shall be combined as a single pooled serum.

(iii) If less than seven rabbits are available, the test is invalid and shall be repeated: Provided, That, if the test is not repeated, the serial shall be declared unsatisfactory.

(4) The antitoxin content of the rabbit sera shall be determined as follows:

(i) Make a dilution of Standard Antitoxin to contain 1 International Unit of antitoxin per ml.

(ii) Make one dilution of Standard Toxin to contain $10 L_0$ doses per ml and make a second dilution of Standard Toxin to contain $10 L_\partial$ doses per ml.

(iii) Combine 1 International Unit of Standard Antitoxin with $10 L_0$ doses of diluted Standard Toxin and Combine 1 International Unit of Standard Antitoxin with $10 L_\partial$ doses of diluted Standard Toxin.

(iv) Dilute 1 ml of serum with 1 ml of diluent (1:2) and combine 1 ml of this

719