

(2) The antitoxin content of the test sample 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 L₀ doses per ml and make a second dilution of Standard Toxin to contain 10 L₊ doses per ml.

(iii) Dilute 1 ml of the test sample with 49 ml of diluent and combine 1 ml of this dilution with 1 ml of the Standard Toxin diluted to contain 10 L₀ doses.

(iv) Combine 10 International Units of Standard Antitoxin with 10 L₀ doses of diluted Standard Toxin and combine 10 International Units of Standard Antitoxin with 10 L₊ 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.

(vi) Five Swiss white mice, each weighing 16–20 grams, shall be used for each toxin-antitoxin mixture. A dose of 0.2 ml shall be injected intravenously into each mouse. Conclude the test 24 hours post-injection and record all deaths.

(3) *Test Interpretation.* (i) If any mice inoculated with the mixture of 10 International Units of Standard Antitoxin and 10 L₀ 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 the mixture of 10 International Units of Standard Antitoxin and 10 L₊ 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 Clostridium Perfringens Type C Antitoxin diluted 1:50 and 10 L₀ doses of Standard Toxin die, the antitoxin is considered to contain less than 500 International Unit per ml and the serial is unsatisfactory.

[39 FR 16859, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991; 61 FR 51777, Oct. 4, 1996]

§ 113.455 Clostridium Perfringens Type D Antitoxin.

Clostridium Perfringens Type D Antitoxin is a specific antibody product containing antibodies directed against the toxin of *Clostridium perfringens* Type D. Each serial shall be tested as provided in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) Each serial shall meet the applicable general requirements provided in § 113.450.

(b) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested using the toxin-neutralization test for Epsilon Antitoxin provided in this section. Dried products shall be rehydrated according to label directions.

(1) 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 L₀ and L₊ doses of Standard Toxin according to their definitions.

(ii) *L₀dose.* 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₊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 solution 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) The antitoxin content of the test sample 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 L₀ doses per ml and make a second dilution of Standard Toxin to contain 10 L₊ doses per ml.

(iii) Dilute 1 ml of the test sample with 33 ml of diluent and combine 1 ml of this dilution with 1 ml of the Standard Toxin diluted to contain 10 L₀ doses.

(iv) Combine 1 International Unit of Standard Antitoxin with 10 L₀ doses of Standard Toxin and combine 1 International Unit of Standard Antitoxin with 10 L₊ doses of 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.

(vi) Five Swiss white mice, each weighing 16–20 grams, shall be used for each toxin-antitoxin mixture. A dose of 0.2 ml shall be injected intravenously into each mouse. Conclude the test 24 hours post-injection and record all deaths.

(3) Test Interpretation. (i) If any mice inoculated with the mixture of 1 International Unit of Standard Antitoxin and 10 L₀ 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 1 International Unit of Standard Antitoxin and 10 L₊ 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 Clostridium Perfringens Type D Antitoxin diluted 1:34 and 10 L₀ doses of Standard Toxin die, the antitoxin is considered to contain less than 34 International Units per ml and the serial is unsatisfactory.

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§§ 113.456–113.498 [Reserved]

§ 113.499 Products for treatment of failure of passive transfer.

A product for the treatment of failure of passive transfer (FPT) shall contain a specified minimum quantity of IgG per dose and shall be recommended for use only in neonates of the same species as that of antibody origin. A product for oral administration shall not be recommended for use in animals more than 24 hours of age, while one for parenteral administration shall only be recommended for use in neonatal animals. Each serial shall meet the applicable general requirements provided in §113.450 and be tested for potency as provided in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Qualification of an IgG Reference Product.* An IgG Reference Product (reference) shall be a serial of product that is manufactured according to the filed Outline of Production, properly qualified, and used to assess the potency of subsequent product serials, as described in paragraph (c) below. The reference shall be qualified as follows:

(1) At least 20 newborn, colostrum-deprived animals of the species for which the product is recommended shall be randomly selected.

(2) Blood samples shall be taken from each animal.

(3) Each animal shall be administered one dose of reference by the recommended route and shall be observed for 24 hours.

(i) Any adverse reactions shall be recorded.

(ii) The dosage of reference administered to each animal shall be in accordance with label directions. Label directions may indicate a single dosage regardless of weight, in which case the animals in the study shall be at or near the maximum weight for neonates of the species.

(4) After 24 hours, blood samples shall be taken from each animal.

(5) Pretreatment and post treatment serum IgG concentrations shall be concurrently determined for each animal using a radial immunodiffusion (RID) method acceptable to APHIS and described in the filed Outline of Production for the product.