

International Unit of Standard Antitoxin and 0.1 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 0.2 ml undiluted serum with 0.1 Lo dose of Standard Toxin die, the serum is considered to contain less than 0.50 International Units per ml.

(iv) If the single pooled serum from seven or more rabbits contains less than 0.5 International Unit per ml, the serial is unsatisfactory.

[39 FR 16862, May 10, 1974, as amended at 45 FR 40101, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990; 56 FR 37825, Aug. 9, 1991, as amended at 56 FR 66784, 66785, Dec. 26, 1991]

§ 113.109 Clostridium Sordellii Bacterin-Toxoid.

Clostridium Sordellii Bacterin-Toxoid shall be produced from a culture of *Clostridium sordellii* which has been inactivated and is nontoxic. Each serial of biological product containing *Clostridium sordellii* 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 § 113.38.

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

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

(i) *International antitoxin unit*. (I.U.) That quantity of antitoxin which reacts with Lo and L+ doses of Standard Toxin according to their definitions.

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

(v) *Standard toxin*. The toxin preparation which is supplied by or is acceptable to the 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 121 °C for 25 minutes; and storing at 4 °C until used.

(2) Each of at least eight rabbits of a strain acceptable to the Animal and Plant Health Inspection Service, each weighing 4-8 pounds, shall be injected subcutaneously with not more than half of the recommended cattle dose: *Provided*, That, if the product is recommended only for sheep, half of the recommended sheep dose shall be used. A second 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 and tested 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 serums shall be determined by the serum neutralization test as follows:

(i) Make a dilution of Standard Antitoxin to contain 1.0 international unit of antitoxin per ml.

(ii) Make a dilution of Standard Toxin in which 1.0 Lo dose is contained

in a volume of 1 ml or less. Make a second dilution of Standard Toxin in which 1.0 L+ dose is contained in a volume of 1 ml or less.

(iii) Combine 1.0 International Unit Standard Antitoxin with 1.0 Lo dose of diluted Standard Toxin and combine 1.0 International Unit of Standard Antitoxin with 1.0 L+ dose of diluted Standard Toxin. Each mixture is adjusted to a final volume of 2.0 ml with diluent.

(iv) Combine 1.0 Lo dose of diluted Standard Toxin with a 1.0 ml volume of undiluted serum. This mixture is adjusted to a final volume of 2.0 ml with diluent.

(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 72 hours post injection and record all deaths.

(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.

[39 FR 16862, May 10, 1974, as amended at 42 FR 61247, Dec. 2, 1977; 45 FR 40101, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990; 56 FR 37826, Aug. 9, 1991; 56 FR 66784, 66785, Dec. 26, 1991]

§ 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