

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of deaths for a satisfactory test	Cumulative total number of deaths for an unsatisfactory test
1	8	8	1 or less	3 or more.
2	8	16	4 or less	5 or more.

The second stage shall be required only when exactly two animals die in the first stage. The second stage shall be conducted in a manner identical to the first stage.

[39 FR 16862, May 10, 1974, as amended at 45 FR 40100, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990 and amended at 56 FR 66784, 66785, Dec. 26, 1991]

§ 113.107 Clostridium Haemolyticum Bacterin.

Clostridium Haemolyticum Bacterin shall be produced from a culture of *Clostridium haemolyticum* which has been inactivated and is nontoxic. Each serial of biological product containing *Clostridium haemolyticum* 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 two-stage test provided in this paragraph.

(1) Each of at least 8 but not more than 10 guinea pigs, each weighing 300 to 500 grams, shall be injected subcutaneously with a guinea pig dose. A second guinea pig dose shall be injected 21 to 23 days after the first dose. Each guinea pig dose shall be one-fifth of the dose recommended on the label for a calf.

(2) *Clostridium haemolyticum* challenge material, available upon request from Animal and Plant Health Inspection Service, shall be used for challenge 14 to 15 days following the last injection

of the product. Each of eight vaccinates and each of five additional non-vaccinated guinea pigs for controls shall be injected intramuscularly with approximately 100 LD₅₀ of challenge material. This dose shall be determined by statistical analysis of results of titrations of the challenge material. The vaccinates and controls shall be observed for 3 days postchallenge and all deaths recorded.

(3) For a valid test, at least 80 percent of the controls shall die within the 3 day post-challenge observation period. If this requirement is met, the results of the potency test shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of deaths for a satisfactory test	Cumulative total number of deaths for an unsatisfactory test
1	8	8	1 or less	3 or more.
2	8	16	4 or less	5 or more.

The second stage shall be required only when exactly two animals die in the first stage. The second stage shall be conducted in a manner identical to the first stage.

[39 FR 16862, May 10, 1974, as amended at 40 FR 20067, May 8, 1975; 45 FR 40100, June 13, 1980. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66785, Dec. 26, 1991]

§ 113.108 Clostridium Novyi Bacterin-Toxoid.

Clostridium Novyi Bacterin-Toxoid shall be produced from a culture of *Clostridium novyi* which has been inactivated and is nontoxic. Each serial of biological product containing *Clostridium novyi* 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 Alpha 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 Alpha 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 Alpha Antitoxin preparation which has been standardized as to antitoxin unitage on the basis of the International *Clostridium novyi* Alpha 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 Alpha 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 0.1 International Unit of antitoxin per ml.

(ii) Make a dilution of Standard Toxin in which 0.1 Lo dose is contained in a volume of 1 ml or less. Make a second dilution of Standard Toxin in which 0.1 L+ dose is contained in a volume of 1 ml or less.

(iii) Combine 0.1 International Unit of Standard Antitoxin with 0.1 Lo dose of diluted Standard Toxin and combine 0.1 International Unit of Standard Antitoxin with 0.1 L+ dose of diluted Standard Toxin. Each mixture is adjusted to a final volume of 2.0 ml with diluent.

(iv) Combine 0.1 Lo dose of diluted Standard Toxin with a 0.2 ml volume of undiluted serum. The 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 0.1 International Unit of Standard Antitoxin and 0.1 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 0.1

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.

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§ 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