§ 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.

(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 $L_0$ 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 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 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 LD₉₀ doses per ml and make a second dilution of Standard Toxin to contain 10 LD₉₀ doses per ml.
(iii) Combine 1 International Unit of Standard Antitoxin with 10 LD₉₀ doses of diluted Standard Toxin and Combine 1 International Unit of Standard Antitoxin with 10 LD₉₀ doses of diluted Standard Toxin.
(iv) Dilute 1 ml of serum with 1 ml of diluent (1:2) and combine 1 ml of this solution with 10 LD₉₀ 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.

(5) Test Interpretation shall be as follows:

(i) If any mice inoculated with the mixture of 1 International Unit of Standard Antitoxin and 10 LD₉₀ doses of Standard Toxin die, 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 LD₉₀ 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 LD₉₀ doses of Standard Toxin die, the serum is considered to contain less than 2 International Units per ml, and the serial is unsatisfactory.