

for such fraction(s) that has been shown to be antigenic or immunogenic in accordance with paragraph (c)(2)(iv)(A) of this section.

[57 FR 38756, Aug. 27, 1992, as amended at 59 FR 67616, Dec. 30, 1994; 64 FR 43044, Aug. 9, 1999; 67 FR 15714, Apr. 3, 2002]

§ 113.114 Tetanus Toxoid.

Tetanus Toxoid shall be produced from a culture of *Clostridium tetani* which has been inactivated and is nontoxic. The toxoid may be either absorbed, precipitated, or purified and concentrated. Each serial of biological product containing *tetanus toxoid* 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 or subserial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product from each serial and 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. A group of 10 guinea pigs consisting of an equal number of males and females weighing 450 to 550 grams shall each be injected subcutaneously with 0.4 of the largest dose recommended on the product labels.

(1) Six weeks after injection, all surviving guinea pigs shall be bled and equal portions of serum, but not less than 0.5 ml from each, shall be pooled. For a valid test, the pool shall contain the serum from at least eight animals.

(2) The antitoxin titer of the pooled serum shall be determined in antitoxin units (A.U.) per ml using an enzyme-linked immunosorbent assay method acceptable to the Animal and Plant Health Inspection Service.

(3) If the antitoxin titer of the serum pool is at least 2.0 A.U. per ml, the serial is satisfactory. If the antitoxin titer of the serum pool is less than 2.0 A.U. per ml, the serial may be retested by the following procedure: *Provided,*

That, if the serial is not retested, it shall be declared unsatisfactory.

(4) For serials in which the serum pool contains less than 2.0 A.U. per ml, the individual serum that constituted the pool may be tested by the enzyme-linked immunosorbent assay. If at least 80 percent of the individual serums have an antitoxin titer of at least 2.0 A.U. per ml, the serial is satisfactory. If less than 80 percent of the individual serums have an antitoxin titer of at least 2.0 A.U. per ml, the serial may be retested in 10 guinea pigs using the procedure described in (c)(1) and (2) above. The antitoxin titer of the pooled serum from the guinea pigs used in the retest shall be averaged with the antitoxin level of the pooled serum from the initial test. If the average of the two pools is at least 2.0 A.U. per ml, the serial is satisfactory. If the average of the two pools is less than 2.0 A.U. per ml, the serial is unsatisfactory and shall not be retested further.

[39 FR 16862, May 10, 1974, as amended at 46 FR 23224, Apr. 24, 1981; 50 FR 24905, June 14, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 37827, Aug. 9, 1991; 56 FR 66785, Dec. 26, 1991]

§ 113.115 Staphylococcus Aureus Bacterin-Toxoid.

Staphylococcus Aureus Bacterin-Toxoid shall be prepared from toxoided broth cultures of selected toxogenic strains of *Staphylococcus aureus* which has been inactivated and is nontoxic. Each serial of biological product containing Staphylococcus Aureus Bacterin-Toxoid 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 shall be tested for viable bacteria and fungi as provided in § 113.26.

(b) *Safety test.* Bulk or final container samples of completed product shall be tested for safety as provided in § 113.33(b). Also, the rabbits used in the potency test provided in paragraph (c) of this section shall constitute an additional safety test. If unfavorable reactions attributable to the product occur

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in any of the rabbits during the observation period, the serial is unsatisfactory.

(c) *Potency test.* Rabbits, each weighing 2000-3000 grams, shall be used as test animals. Either a five rabbit individual serum test or an eight rabbit pooled serum test shall be conducted. At the start of the test, individual serums from the five rabbits or pooled serums from the eight rabbits shall contain less than 0.2 alpha antitoxin units per ml.

(1) Each rabbit shall be given a series of not more than three intramuscular injections at 7 day intervals (1.0 ml, 2.0 ml, 3.0 ml) and observed from 7-14 days following the third injection. At the end of the observation period, a blood sample shall be taken from each rabbit.

(2) The sample of serum from each rabbit, if the five rabbit individual test is conducted or a pooled sample of equal quantities of serum from the rabbits if the eight rabbit pooled serum test is conducted, shall be tested to determine the staphylococcus alpha antitoxin units per ml as provided in paragraphs (c)(3), (4), (5), (6), (7), and (8) of this section.

(3) Inactivate rabbit serum 56 °C for 30 minutes.

(4) Make serial twofold dilutions of the serum samples and conduct the test, using 1 ml of the serial dilutions. Appropriate controls should be included for accurate interpretations.

(5) Add 1 ml of the standardized toxin containing the established "Lh" dose. The "Lh" dose is the amount of toxin which when mixed with one unit of standard antitoxin produces a 50 percent hemolysis of rabbit red blood cells.

(6) Incubate toxin-antitoxin mixture at room temperature for 30 minutes and add 1 ml of a 1.5 percent suspension of washed freshly drawn rabbit red blood cells suspended in normal saline to each tube. Mix and incubate the combined product in a 37 °C water bath for 1 hour. Refrigerate at 5 °C overnight.

(7) Read the hemolysis produced and establish the 50 percent end point. The 50 percent end point of hemolysis should be established by determining the size of the button produced by the unlysed red blood cells.

(8) Determine the units of antitoxin per 1 ml of serum.

(9) If the individual samples from four of the five rabbits in the individual serum test or the pooled samples from the eight rabbits in the pooled serum test do not contain three alpha antitoxin units per ml, the serial is unsatisfactory.

[39 FR 16862, May 10, 1974. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.116 *Pasteurella Multocida* Bacterin, Avian Isolate, Type 4.

Pasteurella Multocida Bacterin, Avian Isolate, Type 4 shall be prepared from cultures of *Pasteurella multocida*, avian isolate, Type 4 (Little and Lyons classification), which have been inactivated, and are nontoxic. Each serial of biological product containing *Pasteurella Multocida* Bacterin, Avian Isolate, Type 4, 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 shall be tested for viable bacteria and fungi as provided in 9 CFR 113.26.

(b) *Safety test.* Observation of the vaccinated turkeys during the prechallenge period of the potency test provided in paragraph (c) of this section shall constitute the safety test. If unfavorable reactions that are attributable to the product occur, the serial is unsatisfactory. If unfavorable reactions that are not attributable to the product occur in one turkey, test results shall be determined by observing the remaining 20 turkeys. The test is inconclusive and may be repeated if unfavorable reactions that are not attributable to the product occur in two or more turkeys, but the serial is unsatisfactory if the test is not repeated.

(c) *Potency test.* Bulk or final container samples of completed product shall be tested for potency of the Type 4 strain, using the two-stage test provided in this paragraph. Turkeys at least 6 weeks old obtained from the same source and hatch shall be properly identified and used as provided in this paragraph.