same manner as the plates containing samples of the serial. All plates shall be examined at the end of the incubation period. If growth is observed on the negative control plate, or no growth is observed on the positive control plate, the test shall be considered inconclusive and may be repeated. If the average number of bacterial colonies on the 10 plates containing samples of the serial exceeds that specified in the filed Outline of Production for the product, one retest to rule out faulty technique may be conducted on samples from 20 final containers. If the average number of bacterial colonies on the retest plates exceeds that specified in the filed Outline of Production for the product, or if a retest is not initiated within 21 days of the completion of the original test, the serial or subserial is unsatisfactory.

(i) *Safety tests.* Bulk or final container samples of each serial shall be tested as prescribed in § 113.33(b). Dried product shall be reconstituted as indicated on the label and 0.5 ml injected per mouse.

[61 FR 51774, Oct. 4, 1996]

§ 113.451 Tetanus Antitoxin.

Tetanus Antitoxin is a specific antibody product containing antibodies directed against the toxin of *Clostridium tetani*. Each serial shall meet the applicable general requirements provided in § 113.450 and paragraph (a) of this section, and be tested for potency as provided in paragraph (b) of this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *General requirements.* The amount of antitoxin in a final container shall be the amount which is delivered from such container when opened and inverted until the flow stops. A graduated volumetric cylinder which conforms to the National Institute of Standards and Technology requirements shall be used. The reading shall be made at the bottom of the meniscus. Volumes of 10 ml or less shall be recorded to the nearest 0.1 and volumes over 10 ml shall be recorded to the nearest ml.

(1) All final containers of Tetanus Antitoxin shall yield not less than the labeled unitage of antitoxin throughout the dating period. The minimum package size permitted for marketing in the United States shall be a 1,500 unit vial.

(2) The expiration date of Tetanus Antitoxin shall be not more than 3 years after the date of a potency test which demonstrates that the recoverable antitoxin from the final container provides at least 20 percent excess over the number of units claimed on the label or not more than 1 year after the date of a potency test which demonstrates that the recoverable antitoxin from the final container provides 10 to 19 percent excess over the number of units claimed on the label.

(b) *Potency test.* Bulk or final container samples of completed product from each serial shall be assayed to calculate the units of Tetanus Antitoxin in each final container. A comparative toxin-antitoxin neutralization test shall be conducted using a standard antitoxin and a standard toxin. All dilutions shall be made in M/15 phosphate buffered (pH) 7.4 physiological saline with 0.2 percent gelatin.

(1) One ml of the Standard Antitoxin shall be diluted before use so the final volume contains 0.1 unit per ml. The dilution shall be held at 20° to 25 ° C for 30 minutes prior to combination with a test does of toxin.

(2) The Standard Toxin test dose is that amount which when mixed with 0.1 unit of Standard Antitoxin, incubated at 20° to 25 ° C for 1 hour, and injected subcutaneously into a 340 to 380 gram guinea pig, results in death of that guinea pig within 60 to 120 hours with clinical signs of tetanus. The toxin shall be diluted so the test dose shall be in 2.0 ml.

(3) A mixture of diluted Standard Toxin and diluted Standard Antitoxin shall be made so that 0.1 unit of antitoxin in 1 ml is combined with a test dose of toxin. This Standard Toxin-Antitoxin mixture shall be held at 20° to 25 ° C for 1 hour before injections of guinea pigs are made.

(4) A sample from each serial of antitoxin shall be prepared as was the Standard Toxin-Antitoxin mixture; except the amount of antitoxin shall be based on an estimation of the expected potency. When testing is done on bulk material, the final container fill shall

reflect the endpoint value plus 10 percent overage for 1 year dating and 20 percent overage for 3 year dating.

(5) Normal guinea pigs weighing within a range of 340 to 380 grams shall be used. Pregnant guinea pigs must not be used.

(i) Each of two guinea pigs (controls) shall be injected subcutaneously with a 3 ml dose of the Standard Toxin-Antitoxin mixture. Injections shall be made in the same order that toxin is added to the dilutions of antitoxins. These shall be observed parallel with the titration of one or more unknown antitoxins.

(ii) Two guinea pigs shall be used as test animals for each dilution of the unknown antitoxin. A 3.0 ml dose shall be injected subcutaneously into each animal.

(6) Controls shall be observed until they are down and are unable to rise or stand under their own power. At this time they are euthanized and the time of death is recorded in hours. For a satisfactory test, the controls must reach this point with clinical signs of tetanus within 24 hours of each other and within an overall time of 60 to 120 hours. The clinical signs to be observed are increased muscle tonus, curvature of the spine, asymmetry of the body outline when the resting animal is viewed from above, generalized spastic paralysis, particularly of the extensor muscles, inability to rise from a smooth surface when the animal is placed on its side, or any combination of these signs. If the control guinea pigs do not respond in this manner, the entire test shall be repeated.

(7) Potency of an unknown antitoxin is determined by finding the mixture which will protect the test animal the same as the Standard Toxin-Antitoxin mixture. Test animals dying sooner than the controls indicate the unit value selected in that dilution was not present, whereas those living longer indicate a greater unit value.

[39 FR 16859, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974, and amended at 40 FR 760, Jan. 3, 1975; 40 FR 41996, Sept. 10, 1975; 43 FR 1479, Jan. 10, 1978; 50 FR 24905, June 14, 1985. Redesignated at 55 FR 35561, Aug. 31, 1990; 61 FR 51776, Oct. 4, 1996; 64 FR 43045, Aug. 9, 1999]

§ 113.452 Erysipelothrix Rhusiopathiae Antibody.

Erysipelothrix Rhusiopathiae Antibody is a specific antibody product containing antibodies directed against one or more somatic antigens of *Erysipelothrix rhusiopathiae*. 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 two-stage test provided in this section.

(1) In the first stage, each of 40 Swiss mice, each weighing 16 to 20 grams, shall be injected subcutaneously with 0.1 ml of product (dried product shall be rehydrated according to label directions). Twenty-four hours postinjection, the injected mice and 10 additional mice designated controls shall be challenged subcutaneously with the same culture of *Erysipelothrix rhusiopathiae*.

(2) If less than eight of the 10 controls die from erysipelas within 7 days post-challenge, the test is invalid. All dead mice shall be examined to determine if the cause of death was *Erysipelothrix rhusiopathiae* infection.

(3) The mice injected with product shall be observed for 10 days postchallenge and all deaths recorded. The second stage shall be required when 7–10 of the mice injected with product die in the first stage. The second stage shall be conducted in a manner identical to the first stage.

(4) The results of the 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	40	40	6 or less	11 or more.