shall be tested for potency of the Type 3 strain, using the two-stage test provided in this paragraph. Turkeys, at least 6 weeks of age, obtained from the same source and hatch, shall be properly identified and used as provided in this paragraph.

(1) *Vaccinates.* Each of not more than 21 turkeys shall be injected with the dose and by the route recommended on the label. A second dose shall be injected after 3 weeks and the turkeys observed for an additional 2 week prechallenge period.

(2) *Unvaccinated controls.* Each of not more than 11 turkeys shall be held as controls.

(3) *Challenge.* Not less than 14 days after the second injection, each of 20 vaccinates, and each of 10 unvaccinated controls shall be challenged intramuscularly with a minimum of 150 colony-forming units of virulent *Pasteurella multocida,* Strain P–1059, Type 3 (Little and Lyons Classification) and observed daily for a 14-day postchallenge period. Only dead birds shall be considered in evaluating the product.

(4) *Validity requirements.* Eight or more unvaccinated controls must die for the test to be valid. If these requirements are met, the potency test results are evaluated according to stage one of the following table. The test is inconclusive and may be repeated if the validity requirements are not met, but the serial is unsatisfactory if the test is not repeated.

Stage	Number of vaccinates	Cumulative number of vaccinates	Cummulative total number of dead vaccinates for_____	
			Satisfactory serial	Unsatisfactory serial
1	20	20	6 or less	9 or more.
2	20	40	15 or less ...	16 or more.

(5) The serial shall pass or fail based on the stage one results of the potency test. However, the second stage may be conducted if seven or eight vaccinates die in stage one, but the serial is unsatisfactory if the second stage is not conducted.

(6) The second stage shall be conducted in a manner identical to the first stage. The serial shall be evaluated according to stage two of the table. On the basis of accumulated results from the data of both stage tests, a serial shall either pass or fail the second stage.

[39 FR 16862, May 10, 1974, as amended at 40 FR 759, Jan. 3, 1975; 47 FR 5196, Feb. 4, 1982; 52 FR 9118, Mar. 23, 1987. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

§ 113.119 Erysipelothrix Rhusiopathiae Bacterin.

Erysipelothrix Rhusiopathiae Bacterin shall be produced from a culture of *Erysipelothrix rhusiopathiae* which has been inactivated and is nontoxic. Each serial of biological product containing *Erysipelothrix rhusiopathiae* 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 mouse protection test provided in this paragraph. A mouse dose shall be 1/10 of the least dose recommended on the label for swine. Such swine dose shall not be less than 1 ml.

(1) The ability of the bacterin being tested (Unknown) to protect mice shall be compared with a Standard Reference Bacterin (Standard) which is either supplied by or acceptable to Animal and Plant Health Inspection Service.

(2) At least three threefold dilutions shall be made with the Standard and the same threefold dilutions shall be made for each Unknown. Dilutions shall be made with physiological saline solution.

(3) For each dilution of the Standard and each dilution of an Unknown, a group of at least 20 mice, each weighing 16 to 22 grams, shall be used. Each mouse in each group shall be injected subcutaneously with one mouse dose of the appropriate dilution.

(4) Each of 20 injected mice from each group shall be challenged subcutaneously 14 to 21 days after being injected. A dose containing at least 100 mouse LD_{50} of a suitable culture of *Erysipelothrix rhusiopathiae* shall be used. All survivors in each group of mice shall be recorded 10 days postchallenge.

(5) Test for valid assay: At least two dilutions of the Standard shall protect more than 0 percent and two dilutions shall protect less than 100 percent of the mice injected. The lowest dilution of the Standard shall protect more than 50 percent of the mice. The highest dilution of the Standard shall protect less than 50 percent of the mice.

(6) The relative potency (RP) of the Unknown is determined by comparing the 50 percent endpoint dilution (highest bacterin dilution protecting 50 percent of the mice) of the Unknown with that of the standard by the following formula:

$$RP = \frac{\text{reciprocal of 50 percent endpoint dilution of Unknown}}{\text{reciprocal of 50 percent endpoint dilution of Standard}}$$

(7) If the RP of the Unknown is less than 0.6, the serial being tested is unsatisfactory.

(8) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the lowest dilution does not exceed 50 percent protection, that serial may be retested in a manner identical to the initial test: *Provided*, That, if the Unknown is not retested or if the protection provided by the lowest dilution of the Standard exceeds the protection provided by the lowest dilution of the Unknown by six mice or more; or, if the total number of mice protected by the Standard exceeds the total number of mice protected by the Unknown by eight mice or more, the serial is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than 0.6, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP values obtained in the retests shall be determined. If the average RP is less than 0.6, the serial is unsatisfactory without further testing. If the average RP obtained in the retests is equal to or greater than 0.6, the following shall apply:

(i) If the RP obtained in the original test is one-third or less than the average RP obtained in the retests, the initial RP may be considered a result of test system error and the serial is satisfactory for potency.

(ii) If the RP value obtained in the original test is more than one-third the average RP obtained in the retests, a new average shall be determined using the RP values obtained in all tests. If the new average is less than 0.6, the serial is unsatisfactory.

[39 FR 16862, May 10, 1974, as amended at 40 FR 759, Jan. 3, 1975; 40 FR 20067, May 8, 1975; 40 FR 51414, Nov. 5, 1975; 44 FR 71408, Dec. 11, 1979; 50 FR 23795, June 6, 1985; 51 FR 23731, July 1, 1986. Redesignated at 55 FR 35562, Aug. 31, 1990; 56 FR 66558, Dec. 24, 1991; 56 FR 66784, 66785, Dec. 26, 1991]

§ 113.120 Salmonella Typhimurium Bacterin.

Salmonella Typhimurium Bacterin shall be prepared from a culture of *Salmonella typhimurium* which has been inactivated and is nontoxic. Each serial of biological product containing *Salmonella typhimurium* 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 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 mouse test provided in this paragraph. A mouse dose shall be ½₀ of the least dose recommended on the label for other animals which shall not be less than 2 ml.