the minimum or greater than the maximum required in paragraph (b)(2) of this section, the serial or subserial is unsatisfactory.

(4) If tested at any time within the expiration period, each dose of rehydrated vaccine must contain at least 3.0 billion viable organisms per dose.

(c) *Bacterial count requirements for standard vaccine.* Each serial and subserial shall be tested for potency.

(1) Two final container samples shall be tested for the number of viable organisms per milliliter of rehydrated vaccine. One bacterial count per vial shall be made on tryptose agar plates from suitable dilutions using 1 percent peptone as a diluent. The inoculated media shall be incubated at 35 to 37 ° C for 96 hours.

(2) If the average count of the two final container samples of freshly prepared vaccine does not contain at least 10 billion viable organisms per milliliter, the serial or subserial is unsatisfactory.

(3) If the initial bacterial count is less than 10 billion organisms per milliliter, the serial or subserial may be retested one time using four samples. If the average count of the four vials retested is less than the required minimum, the serial or subserial is unsatisfactory.

(4) If tested at any time within the expiration period, each milliliter of rehydrated vaccine does not contain at least 5 billion viable organisms per milliliter, the serial or subserial is unsatisfactory.

[39 FR 16857, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974, and amended at 40 FR 758, Jan. 3, 1975; 50 FR 23794, Jan. 6, 1985]

§ 113.66 Anthrax Spore Vaccine—Nonencapsulated.

Anthrax Spore Vaccine—Nonencapsulated shall be a live spore suspension prepared from nonencapsulated variants of *Bacillus anthracis*. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.64 and the requirements in this section.

(b) Each lot of Master Seed shall be tested for immunogenicity as follows:

(1) Forty-two susceptible guinea pigs from the same source each weighing 400 to 500 grams, shall be used as test animals (30 vaccinates and 12 controls).

(2) An arithmetic mean spore count of vaccine produced from the highest passage of the Master Seed shall be established before the immunogenicity test is conducted. The guinea pigs used as vaccinates shall be injected as recommended on the label with a predetermined number of vaccine spores. To confirm the dosage, five replicate spore counts shall be conducted on a sample of the vaccine dilution used.

(3) Fourteen to fifteen days postvaccination the vaccinates and controls shall each be challenged with not less than 4,500 guinea pig LD_{50} of a virulent suspension of *Bacillus anthracis* furnished or approved by Animal and Plant Health Inspection Service and observed for 10 days.

(4) If at least 10 of the 12 controls do not die from *Bacillus anthracis* within the 10-day postchallenge observation period the test is invalid and may be repeated.

(5) If at least 27 of 30 of the vaccinates do not survive the 10-day postchallenge observation period, the Master Seed is unsatisfactory.

(6) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. The vaccinates and controls must meet the criteria prescribed in paragraphs (b)(4) and (b)(5) of this section.

(7) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(c) *Test Requirements for Release.* Each serial and subserial shall meet the applicable general requirements prescribed in 9 CFR 113.64 and the requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* Samples of completed product from each serial or first subserial shall be tested for safety in sheep

or goats by the methods described in 9 CFR 113.45(a).

(2) *Spore Count Requirements.* Final container samples of completed product shall be tested for spore count. Samples shall be diluted in tenfold steps. Each dilution expected to yield 30 to 300 colonies per plate shall be plated in triplicate on tryptose agar, inverted, and incubated at 35 to 70 °C for 24 hours to 28 hours. Each plate having uniformly distributed colonies shall be counted and an average count determined. To be eligible for release, each serial and each subserial shall have a spore count sufficiently greater than that of the vaccine used in the immunogenicity test to assure that when tested at any time within the expiration period, each serial and subserial shall have a spore count of at least twice that used in the immunogenicity test but not less than 2,000,000 spores per dose.

[50 FR 23794, June 6, 1985, as amended at 56 FR 66784, Dec. 26, 1991]

§ 113.67 Erysipelothrix Rhusiopathiae Vaccine.

Erysipelothrix Rhusiopathiae Vaccine shall be prepared as a desiccated live culture of an avirulent or modified strain of *Erysipelothrix rhusiopathiae.* Only Master Seed which has been established as pure, safe, and immunogenic shall be used for vaccine production.

(a) The Master Seed shall meet the applicable requirements prescribed in § 113.64 and the requirements in this section.

(b) Each lot of Master Seed used for vaccine production shall be tested for immunogenicity. The selected bacterial count from the lot of Master Seed shall be established as follows:

(1) Thirty *Erysipelothrix rhusiopathiae* susceptible swine shall be used as test animals (20 vaccinates and 10 controls) for each route of administration recommended on the label.

(2) An arithmetic mean count of the colony forming units from vaccine produced from the highest passage of the Master Seed shall be established before the immunogenicity test is conducted. The 20 swine to be used as vaccinates shall be injected as recommended on the label with a predetermined quantity of vaccine bacteria. The 10 control swine shall be held separately from the vaccinates. To confirm the dosage calculation, an arithmetic mean count shall be established by conducting five replicate titrations on a sample of the bacterial vaccine dilution used. Only plates containing between 30 and 300 colonies shall be considered in a valid test.

(3) The vaccinates and controls shall be examined and their average body temperature determined prior to challenge. Fourteen to twenty-one days postvaccination, the vaccinates and controls shall be challenged with a virulent *Erysipelothrix rhusiopathiae* culture and observed for 7 days. The challenge culture and instructions for preparation and use shall be obtained from Animal and Plant Health Inspection Service.

(4) A satisfactory challenge shall be evidenced in the controls by a high body temperature or clinical signs including, but not limited to acute illness with hyperemia of the abdomen and ears, possibly terminating in sudden death; moribundity, with or without metastatic skin lesions; depression with anorexia, stiffness, and/or joint involvement; or any combination of these symptoms and lesions.

(5) If at least 80 percent of the controls do not show characteristic signs during the observation period including, but not limited to a body temperature of 105.6 ° F or higher on at least 2 consecutive days, the test shall be considered inconclusive: *Provided,* That control pigs which meet the criteria requirements for susceptibility except for high body temperature shall be considered susceptible if sacrificed and organisms identified as *Erysipelothrix rhusiopathiae* can be isolated from the blood, spleen, or other organs.

(6) To demonstrate immunity after challenge, the vaccinates shall remain free of clinical signs and the body temperature shall not exceed 104.6 ° F on 2 or more consecutive days. If at least 90 percent of the vaccinates do not remain free from clinical signs and high body temperature throughout the observation period, the Master Seed is unsatisfactory.

(7) The Master Seed shall be retested for immunogenicity in 3 years. Only