

(c) *Identity test.* At least one of the identity tests provided in this paragraph shall be conducted for the Master Seed Bacteria and final container samples from each serial or first subserial of completed biological product. A known positive control (reference) provided or approved by Animal and Plant Health Inspection Service shall be included in such tests.

(1) *Fluorescent antibody test.* The direct fluorescent antibody staining technique shall be conducted using suitable smears of the vaccine bacteria. Fluorescence typical for the bacteria concerned shall be demonstrated. Fluorescence shall not occur in control smears treated with specific antiserum.

(2) *Tube agglutination test.* A tube agglutination test shall be conducted with a suitable suspension of the vaccine bacteria using the constant antigen decreasing serum method with specific antiserum. Agglutination typical for the bacteria shall be demonstrated. Agglutination shall not occur with negative serum used as a control in this test.

(3) *Slide agglutination test.* The rapid plate (slide) agglutination test shall be conducted with suitable suspensions of the vaccine bacteria using the hanging drop, slide or plate method, with specific antiserum. Agglutination typical for the bacteria shall be demonstrated by microscopic or macroscopic observation. Agglutination shall not occur with negative serum used as a control in this test.

(4) *Characterization tests.* Applicable biochemical and cultural characteristics shall be demonstrated as specified in the filed Outline of Production.

(d) *Ingredient requirements.* Ingredients used for the growth and preparation of Master Seed Bacteria and of live bacterial vaccine shall meet the requirements provided in § 113.50. Ingredients of animal origin shall meet the applicable requirements provided in § 113.53.

(e) *Moisture content.* The maximum percent moisture in desiccated vaccines shall be stated in the filed Outline of Production and shall be established by the licensee as follows:

(1) *Prelicensing.* Data obtained by conducting accelerated stability tests and

bacterial counts shall be acceptable on a temporary basis.

(2) *Licensed products.* Data shall be obtained by determining the percent moisture and bacterial count at release and expiration on a minimum of 10 consecutive released serials.

(3) Final container samples of completed product from each serial and subserial must be tested for moisture content in accordance with the test provided in § 113.29.

[48 FR 33476, July 22, 1983, as amended at 54 FR 19352, May 5, 1989; 56 FR 66784, Dec. 26, 1991; 68 FR 57608, Oct. 6, 2003]

§ 113.65 *Brucella Abortus Vaccine.*

Brucella Abortus Vaccine shall be prepared as a desiccated live culture bacterial vaccine from smooth colonial forms of the *Brucella abortus* organism, identified as Strain 19. Each serial and subserial shall be tested for purity, potency, and moisture content. A serial or subserial found unsatisfactory by a prescribed test shall not be released.

(a) *Purity tests.* Each serial and subserial shall be tested for purity as provided in this paragraph.

(1) Macroscopic and microscopic examination shall be made on bulk samples from production containers. If organisms not typical of *Brucella abortus* organisms are evident, the serial or subserial is unsatisfactory.

(2) Two final container vials of completed product shall be tested by inoculating one tube of Dextrose Andrades broth with gas tube and one tube of thioglycollate broth from each vial. The inoculated media shall be incubated at 35 to 37 ° C for 96 hours. If growth not typical of *Brucella abortus* organisms is evident, the serial or subserial is unsatisfactory.

(3) Bacterial dissociation test. Final container samples of completed product from each serial and subserial shall be tested for bacterial dissociation. Smooth colonies are the desired form. Rough colonies are undesirable terminal dissociation forms. Intermediate and intermediate-to-rough are also undesirable.

(i) The sample container shall be rehydrated and streaked on one potato agar plate in such a manner as to produce confluent colonies. Artificial reflected light shall be used so that the

rays pass through the plate at a 45° angle.

(ii) If the vaccine contains more than 5 percent rough colonies or more than 15 percent total undesirable colonies, the serial or subserial is unsatisfactory. If organisms or growth not characteristic of *Brucella abortus* are found, the serial or subserial is unsatisfactory. The test may be repeated one time using double the number of samples: *Provided*, That, if the test is not repeated, the serial or subserial is unsatisfactory.

(b) *Bacterial count requirements for reduced dose vaccine.* Each serial and each subserial shall be tested for potency.

(1) Two final container vials of completed product shall be tested for the number of viable organisms per dose of rehydrated vaccine. A 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 contains less than 3.0 or more than 10.0 billion organisms per dose, the serial or subserial is unsatisfactory.

(3) If the average count on the initial test is less than the minimum or greater than the maximum required in paragraph (b)(2) of this section, the serial or subserial may be retested one time using four additional final container vials. The average count of the retest is determined. If the average count of the four vials retested is less than the required minimum or greater than the required maximum, the serial or subserial is unsatisfactory. If the average count of the four vials retested is within the required limits described in paragraph (b)(2) of this section, the following shall apply:

(i) If the average count obtained in the initial test is less than one-third or more than three times the average count obtained on the retest, the average count of the initial test shall be considered the result of test system error and the serial or subserial is satisfactory.

(ii) If the average count obtained in the initial test is one-third or more than the average retest count or three

times or less than the average retest count, a new average count shall be determined from the counts of all six vials. If the new average is less than 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—Non-encapsulated.

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