

tested for the presence of porcine parvovirus by the fluorescent antibody technique as prescribed in § 113.47(c).

(e) A sample of serum from each donor horse used to produce a lot of equine serum used in the preparation of biological products recommended for use in horses shall be tested at a laboratory approved by Animal and Plant Health Inspection Service using the Coggins test for equine infectious anemia antibodies. If antibodies to equine infectious anemia are found, the lot of serum is unsatisfactory.

[50 FR 442, Jan. 4, 1985; 50 FR 3316, Jan. 24, 1985, as amended at 56 FR 66784, Dec. 26, 1991; 60 FR 24549, May 9, 1995]

#### § 113.54 Sterile diluent.

Sterile Diluent shall be supplied in a final container by the licensee when such diluent is required for rehydration or dilution of the vaccine.

(a) Sterile Diluent may be distilled or deionized water or it may be a special liquid solution formulated in accordance with an acceptable outline on file with Animal and Plant Health Inspection Service.

(b) Each quantity prepared at one time in a single container and bottled into final containers shall be designated as a serial. Each serial shall be given a number which shall be used in records, test reports, and on the final container label.

(c) Final container samples from each serial shall be tested for bacteria and fungi in accordance with the test provided in § 113.26. Any serial found to be unsatisfactory shall not be released.

[39 FR 27428, July 29, 1974, as amended at 56 FR 66784, Dec. 26, 1991]

#### § 113.55 Detection of extraneous agents in Master Seed Virus.

Unless otherwise prescribed in a Standard Requirement or in a filed Outline of Production, each Master Seed Virus (MSV) shall be tested as prescribed in this section. A MSV found unsatisfactory by any prescribed test shall not be used. A serial of biological product shall not be released if produced from a MSV that is found unsatisfactory by any prescribed test.

(a) At least a 1.0 ml aliquot per cell culture of MSV shall be dispensed onto monolayers (at least 75 cm<sup>2</sup> in area) of:

(1) Vero (African green monkey kidney) cell line;

(2) Embryonic cells, neonatal cells, or a cell line of the species for which the vaccine is recommended; and

(3) Embryonic cells, neonatal cells, or a cell line of the species of cells in which the MSV is presently being propagated if different than prescribed in paragraphs (a)(1) and (a)(2) of this section. Cell lines used shall have been found satisfactory when tested as prescribed in § 113.52 and primary cells used shall have been found satisfactory when tested as prescribed in § 113.51. If the MSV is cytopathic for or causes hemadsorption in the cells in which it is to be tested, the MSV shall be neutralized with monospecific antiserum supplied or approved by Animal and Plant Health Inspection Service (APHIS) or counteracted by a method approved by APHIS.

(b) At least one monolayer of each cell type used in the test shall be maintained as an uninoculated control.

(c) Each monolayer shall be maintained at least 14 days.

(d) Cells shall be subcultured at least once during the maintenance period. All but the last subculture shall result in at least one new monolayer at least 75 cm<sup>2</sup>. The last subculture shall meet the minimum area requirement specified in §§ 113.46 and 113.47.

(e) Monolayers shall be examined regularly throughout the 14-day maintenance period for evidence of cytopathogenic agents. If evidence of a cytopathogenic agent is found, the MSV is unsatisfactory.

(f) At the conclusion of the 14-day maintenance period, monolayers shall be tested for:

(1) Cytopathogenic and/or hemadsorbing agents as prescribed in § 113.46;

(2) Extraneous agents by the fluorescent antibody technique as prescribed in § 113.47.

[50 FR 444, Jan. 4, 1985, as amended at 56 FR 66784, Dec. 26, 1991]

#### LIVE BACTERIAL VACCINES

#### § 113.64 General requirements for live bacterial vaccines.

When prescribed in an applicable Standard Requirement or in the filed

Outline of Production, a live bacterial vaccine shall meet the requirements in this section.

(a) *Purity test.* Final container samples of completed product from each serial and subserial, and samples of each lot of Master Seed Bacteria shall be tested for the presence of extraneous viable bacteria and fungi in accordance with the test provided in § 113.27(b).

(b) *Safety tests.* (1) Samples of completed product from each serial or first subserial and samples of each lot of Master Seed Bacteria shall be tested for safety in young adult mice in accordance with the test provided in § 113.33(b) unless:

(i) The bacteria or agents in the vaccine are inherently lethal for mice.

(ii) The vaccine is recommended for poultry.

(2) Samples of completed product from each serial or first subserial of live bacterial vaccine shall be tested for safety in one of the species for which the product is recommended as follows:

(i) Live bacterial vaccine recommended for use in dogs shall be tested as provided in § 113.40, except that dogs shall be injected with the equivalent of two doses of vaccine administered as recommended on the label.

(ii) Live bacterial vaccine recommended for use in cattle shall be tested as provided in § 113.41, except that calves shall be injected with the equivalent of two doses of vaccine administered as recommended on the label.

(iii) Live bacterial vaccine recommended for use in sheep shall be tested as provided in § 113.45.

(iv) Live bacterial vaccine recommended for use in swine shall be tested as provided in § 113.44.

(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.