

of vaccine virus by the method to be recommended on the label. To confirm the dosage administered, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(4) Twenty-one to twenty-eight days postvaccination, blood samples shall be drawn from all test animals. For a valid test, the controls shall remain seronegative at 1:2 final serum dilution. In a valid test, if at least 19 of 20 vaccinates do not have antibody titers of at least 1:20 in a hemagglutination-inhibition test or at least 1:40 in a serum neutralization test, the Master Seed is unsatisfactory.

(5) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot is discontinued. Only five vaccinates and two controls need to be used in the retest: *Provided*, That five of five vaccinates and the two controls shall meet the criteria in paragraph (b)(4) of this section.

(6) 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 §113.300 and special requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* The mouse safety test prescribed in §113.33(b) shall be conducted.

(2) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the method in paragraph (b)(3) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of the vaccine used in the immunogenicity test prescribed in paragraph (b) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer of  $10^{0.7}$  greater than that used in the immunogenicity test, but not less than  $10^{2.5}$  TCID<sub>50</sub> per dose.

[50 FR 23797, June 6, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

### § 113.309 Bovine Parainfluenza<sub>3</sub> Vaccine.

Bovine Parainfluenza<sub>3</sub> Vaccine shall be produced from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the tenth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in §113.300.

(b) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Twenty-five bovine parainfluenza, susceptible calves shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individual serums tested. Also, nasal specimens shall be collected for virus isolation attempts. The calves shall be considered susceptible if:

(i) The results are negative at a 1:2 final serum dilution in a varying serum constant virus neutralization test with less than 500 TCID<sub>50</sub> of bovine parainfluenza<sub>3</sub> virus; and

(ii) Shall be negative to bovine parainfluenza<sub>3</sub> virus isolation attempts from the nasal specimens on the day of injection.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 calves to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five calves held as uninjected controls. To confirm the dosage calculation, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) The vaccinates and controls shall be examined for clinical signs of respiratory disease and the body temperature taken and recorded on each of the

first 14 consecutive days post-injection. The vaccinates shall be bled on day 6±2 days post-injection.

(4) Three to four weeks post-vaccination, all calves shall be bled for serum antibodies and nasal specimens shall be collected for PI<sub>3</sub> virus isolation. On the same day, all vaccinates and controls shall be given acceptable challenge PI<sub>3</sub> virus titrating at least 10<sup>7.0</sup> TCID<sub>50</sub> per ml and the animals observed for 14 days. Two ml of the challenge virus shall be instilled in each nostril or shall be inhaled as an aerosol suspension. Upon request, challenge virus and instructions shall be furnished by Animal and Plant Health Inspection Service.

(5) Each animal shall be examined for clinical signs of respiratory disease and the body temperature recorded on each of the 14 consecutive days of the post-challenge observation period. Each day for at least the first 10 days post-challenge, nasal specimens for virus isolation attempts shall be taken. All animals shall be bled on day 6±2 days post-challenge, and all animals shall be bled at least once 14 to 28 days post-challenge for serum antibody studies.

(6) Satisfactory Test Criteria:

(i) All virus isolations attempts shall be by culture and at least one subculture in PI<sub>3</sub> susceptible cells for a total of at least 14 days.

(ii) Two to four weeks post-vaccination, at least 19 of the 20 vaccinates shall have PI<sub>3</sub> neutralizing antibody titers of at least 1:4 and all five controls shall be negative at 1:2 dilution. None of the post-vaccination serums collected from the vaccinates on day 6±2 days shall reveal serum neutralization antibody titers of 1:32 or greater based upon final dilution.

(iii) Satisfactory resistance to challenge by vaccinates shall be determined by a significant difference between virus isolation rates from vaccinates and controls. The virus neutralization titers of post-challenge serums and respiratory symptoms and temperatures from all animals shall be considered in the evaluation of the test validity.

(7) Designated animal alternates for test animals showing anamnestic antibody responses (titers 1:32 or greater)

on day 6 serums may be included in the study under the following provisions:

(i) No more than five alternates shall be allowed for the vaccinates and no more than two for the controls.

(ii) Alternates shall be subject to all requirements outlined for the animals for which they are alternates.

(iii) Antibody values from alternate animals may be used only to replace values from up to and including five vaccinates which develop antibody of 1:32 or greater by day 6±2 days post-vaccination or up to and including two controls which develop antibody titers of 1:32 or greater by day 6±2 days post-challenge.

(8) A sequential test procedure may be used in lieu of the 20 calf requirement. A beta value of .05 and a tolerance level of .78 shall be required.

(9) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest; *Provided*, That five of five vaccinates and at least four of the controls shall meet the criteria prescribed in paragraph (c)(6) of this section.

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

(d) Test requirements for release: Each serial and subserial shall meet the applicable general requirements prescribed in §113.300 and the requirements in this paragraph. Final container samples of completed product shall be tested except as prescribed in paragraph (d)(1) of this section. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Purity test*. The test for Brucella contamination prescribed in §113.32 shall be conducted on each batch of primary cells intended for production use.

(2) *Safety test*. The mouse safety test prescribed in §113.33(a) and the calf safety test prescribed in §113.41 shall be conducted.

(3) *Virus titer requirements*. Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for

release, each serial and each subserial shall have a virus titer per dose sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of  $10^{0.7}$  greater than that used in the immunogenicity test but not less than  $10^{2.5}$  TCID<sub>50</sub> per dose.

[39 FR 44719, Dec. 27, 1974, as amended at 40 FR 41089, Sept. 5, 1975; 43 FR 49529, Oct. 24, 1978; 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 60 FR 14357, Mar. 17, 1995]

#### § 113.310 Bovine Rhinotracheitis Vaccine.

Bovine Rhinotracheitis Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the tenth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.300.

(b) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) Twenty-five infectious bovine rhinotracheitis susceptible calves shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individual serums tested. The calves shall be considered susceptible if the results are negative at a 1:2 final serum dilution by the virus plaque reduction method.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. The 20 calves to be used as vaccinates shall be injected with a predetermined quan-

tity of vaccine virus and the remaining five calves held as uninjected controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) At least once during a period of 14 to 28 days post-vaccination, individual serum samples shall be collected for virus-neutralization tests from each of the vaccinates. The test virus shall be 100 to 500 TCID<sub>50</sub> bovine rhinotracheitis virus. Results shall be used in making a determination as prescribed in paragraph (c)(6) of this section.

(4) The vaccinates and the controls shall each be challenged with virulent infectious bovine rhinotracheitis virus and observed for 14 days. The rectal temperature of each animal shall be taken and the presence or absence of respiratory or other clinical signs of bovine rhinotracheitis noted and recorded on each of the 14 consecutive days.

(5) If at least four of the five controls do not show clinical signs of infectious bovine rhinotracheitis and a marked temperature rise to 104.5 °F. or higher post-challenge, the test shall be considered inconclusive and may be repeated.

(6) If less than 19 of the post-injection serum samples tested as prescribed in paragraph (c)(3) of this section show neutralization in all tubes of the 1:2 final serum dilution, or if more than one of the vaccinates show a temperature of 103.5 °F. or higher for 2 or more days, or if more than one of the vaccinates exhibit respiratory or other clinical signs of infectious bovine rhinotracheitis, or both, the Master Seed Virus is unsatisfactory.

(7) A sequential test procedure may be used in lieu of the 20 calf requirement. A beta value of .05 and a tolerance level of .78 shall be required.

(8) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest; *Provided*, That five of five vaccinates and at least four of the five controls shall meet the criteria prescribed in paragraphs (c)(5) and (6) of this section.

(9) An outline of Production change shall be made before authority for use