

Seed Virus shall be established as follows:

(1) Twenty-five dogs, less than 12 weeks of age and free of measles antibody, shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individual serum samples tested. The dogs shall be considered susceptible if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test with less than 500 ID₅₀ of measles virus.

(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. Twenty dogs shall be vaccinated with a predetermined quantity of vaccine virus and the remaining five dogs held as unvaccinated controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) On the day of challenge, serum samples shall be obtained from each vaccinate and individually tested for antibody against canine distemper virus. For a valid test, each vaccinate shall be negative at a 1:4 final serum dilution in varying serum-constant virus neutralization test using less than 500 ID₅₀ of canine distemper virus.

(4) At least 21 days postinoculation, the immunity of the vaccinates and controls shall be challenged by exposure to a uniform dose of aerosolized virulent canine distemper virus. All test dogs shall be observed daily for 21 days postchallenge.

(i) If at least 4 of the 5 controls do not die or show signs of distemper, including a temperature of 104.0 °F. or higher and at least 15 percent weight loss, the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates do not survive without showing a temperature of 104.0 °F. or higher and a weight loss exceeding 15 percent after day 8 postchallenge, the Master Seed Virus is unsatisfactory.

(5) When approved in advance by Animal and Plant Health Inspection Service, a sequential test procedure may be used in lieu of the 20 dog requirement.

A beta value of 0.05 and a tolerance level of 0.78 shall be required.

(6) 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 this section.

(7) 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 general requirements prescribed in §113.300 and the requirements in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety tests.* The dog safety test prescribed in §113.40 and the mouse safety test prescribed in §113.33(a) shall be conducted.

(2) *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 sufficiently greater than the titer of the 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} ID₅₀ per dose.

[40 FR 53001, Nov. 14, 1975, as amended at 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]

§ 113.314 Feline Calicivirus Vaccine.

Feline Calicivirus 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

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vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

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

(b) The Master Seed Virus shall be tested for chlamydial agents as prescribed in § 113.43.

(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) Thirty feline calicivirus susceptible cats shall be used as test animals (20 vaccinates and 10 controls). Throat swabs shall be collected from each cat and individually tested on susceptible cell cultures for the presence of feline calicivirus. Blood samples shall be drawn and individual serum samples tested. The cats shall be considered suitable for use if all swabs are negative for virus isolation and if all serums are negative for calicivirus antibody at the 1:2 final dilution in a 50 percent plaque reduction test or other SN test of equal sensitivity.

(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 cats used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method to be recommended on the label and the remaining 10 cats shall be held as controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used. If two doses are used, five replicate confirming titrations shall be conducted on each dose.

(3) Twenty-one or more days after the final dose of vaccine, the vaccinates and controls shall each be challenged intranasally with a minimum of 100,000 TCID₅₀ or plaque forming units of virulent feline calicivirus furnished or approved by Animal and Plant Health Inspection Service and observed each day for 14 days postchallenge. The rectal temperature of each animal shall be taken and the presence or absence of clinical signs, particularly le-

sions on the oral mucosa, noted and recorded each day.

(i) If less than 8 of 10 controls show clinical signs of feline calicivirus infection other than fever, the test is inconclusive and may be repeated.

(ii) If a significant difference in clinical signs cannot be demonstrated between vaccinates and controls using a scoring system approved by Animal and Plant Health Inspection Service and prescribed in the Outline of Production, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Either 10 vaccinates and 6 controls or 5 vaccinates and 3 controls shall be used in the retest.

(i) If less than 5 of 6 or 3 of 3 of the controls in the retest show clinical signs of feline calicivirus infection other than fever, the test is inconclusive and may be repeated.

(ii) A significant difference in clinical signs shall be demonstrated between vaccinates and controls in a valid test as prescribed in paragraph (c)(3)(ii) of this section.

(5) 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 requirements prescribed in § 113.300 and in this paragraph. Final container samples of completed product shall be tested. 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(a) and the cat safety test prescribed in § 113.39(b) shall be conducted.

(2) *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 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 $10^{2.5}$ TCID₅₀ or plaque forming units per dose.

[44 FR 58899, Oct. 12, 1979; 44 FR 63083, Nov. 2, 1979, as amended at 48 FR 33472, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.315 Feline Rhinotracheitis Vaccine.

Feline 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 fifth passage from the Master Seed Virus.

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

(b) The Master Seed Virus shall be tested for chlamydial agents as prescribed in § 113.43.

(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) Thirty feline rhinotracheitis susceptible cats shall be used as test animals (20 vaccinates and 10 controls). Throat swabs shall be collected from each cat and individually tested on susceptible cell cultures for the presence of feline rhinotracheitis virus. Blood samples shall be drawn and individual serum samples tested. The cats shall be considered suitable for use if all swabs are negative for virus isolation and if all serums are negative for feline rhinotracheitis virus antibody at the 1:2 final dilution in a 50 percent plaque reduction test or other SN test of equal sensitivity.

(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 cats used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method to be rec-

ommended on the label and the remaining 10 cats shall be held as controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used. If two doses are used, five replicate confirming titrations shall be conducted on each dose.

(3) Twenty-one or more days after the final dose of vaccine, the vaccinates and controls shall each be challenged intranasally with a minimum of 100,000 TCID₅₀ or plaque forming units of virulent feline rhinotracheitis virus furnished or approved by Animal and Plant Health Inspection Service and observed each day for 14 days post-challenge. The rectal temperature of each animal shall be taken and the presence of respiratory or other clinical signs of feline rhinotracheitis noted and recorded each day.

(i) If less than 8 of 10 controls show clinical signs of feline rhinotracheitis infection other than fever, the test is inconclusive and may be repeated.

(ii) If a significant difference in clinical signs cannot be demonstrated between vaccinates and controls using a scoring system approved by Veterinary Services and prescribed in the Outline of Production, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Either 10 vaccinates and 6 controls or 5 vaccinates and 3 controls shall be used in the retest.

(i) If less than 5 of 6 or 3 of 3 of the controls in the retest show clinical signs of feline rhinotracheitis infection other than fever, the test is inconclusive and may be repeated.

(ii) A significant difference in clinical signs shall be demonstrated between vaccinates and controls in a valid test as prescribed in paragraph (c)(3)(ii) of this section.

(5) 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 requirements prescribed in § 113.300 and