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(i) A constant virus-carrying serum neutralization test in tissue culture using 100 to 300 TCID<sub>50</sub> of virus shall be used.

(ii) Cats shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(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 injected with a predetermined quantity of vaccine virus and the remaining five cats 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) Fourteen days post-injection, the vaccinates and the controls shall be challenged with virulent feline panleukopenia virus furnished by Animal and Plant Health Inspection Service and the cats observed each day for 14 days.

(i) If at least 80 percent of the controls do not show clinical signs of feline panleukopenia during the observation period, the test is inconclusive and may be repeated. Clinical signs of feline panleukopenia shall include a pronounced leukopenia wherein the white cell count drops to 4,000 or less per cubic mm, or the white cell count drops to less than 25 percent of the normal level established by an average of three or more counts taken prior to challenge.

(ii) If at least 19 of the 20 vaccinates do not survive the observation period without showing clinical signs of feline panleukopenia as described in paragraph (c)(3)(i) of this section, the Master Seed Virus is unsatisfactory.

(4) 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 shall be conducted.

(i) Each of two healthy cats shall be injected with 10 cat doses by the method recommended on the label and the cats observed each day for 14 days.

(ii) If unfavorable reactions attributable to the biological product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated: *Provided*, That, if not repeated, the serial shall be unsatisfactory.

(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<sup>0.7</sup> greater than that used in such immunogenicity test but not less than 10<sup>2.5</sup> TCID<sub>50</sub> per dose.

[39 FR 44716, Dec. 27, 1974, as amended at 40 FR 53378, Nov. 18, 1975; 43 FR 25078, June 9, 1978; 43 FR 41186, Sept. 15, 1978; 44 FR 58900, Oct. 12, 1979; 48 FR 33471, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 72 FR 72564, Dec. 21, 2007]

**§ 113.305 Canine Hepatitis and Canine Adenovirus Type 2 Vaccine.**

Canine Hepatitis Vaccine and Canine Adenovirus Type 2 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 in preparing the production seed virus for vaccine production. All serials shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in §113.300 except that the dog safety test prescribed in §113.40(a) shall be conducted by the intravenous route.

(b) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity by one or both of the following methods:

(1) *Immunogenicity for canine hepatitis.* Twenty-five canine hepatitis susceptible dogs shall be used as test animals (20 vaccinates and 5 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 using 50 to 300 TCID<sub>50</sub> of canine adenovirus.

(i) 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 dogs to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five dogs 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.

(ii) Not less than 14 days postinjection, the vaccinates and the controls shall each be challenged intravenously with virulent infectious canine hepatitis virus furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 14 days.

(A) If at least four of the five controls do not show severe clinical signs of canine hepatitis, the test is inconclusive and may be repeated.

(B) If at least 19 of the 20 vaccinates do not survive without showing clinical signs of infectious canine hepatitis during the observation period, the Master Seed Virus is unsatisfactory.

(2) *Immunogenicity for canine adenovirus Type 2.* Thirty canine adenovirus type 2 susceptible dogs shall be used as test animals (20 vaccinates and 10 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 using 50 to 300 TCID<sub>50</sub> of canine adenovirus.

(i) 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 dogs to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining 10 dogs 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.

(ii) Not less than 14 days postinjection, the vaccinates and the controls shall be challenged by exposure to a nebulized aerosol of virulent canine adenovirus type 2 furnished or approved by the 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 of respiratory or other clinical signs of canine adenovirus type 2 noted and recorded each day.

(A) If at least 6 of 10 controls do not show clinical signs of canine adenovirus type 2 infection other than fever, the test is inconclusive and may be repeated.

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

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

(c) *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) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (b)(1)(i) and/or (b)(2)(i) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer

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of vaccine virus used in the immunogenicity test(s) 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 such immunogenicity test(s) but not less than  $10^{2.5}$  TCID<sub>50</sub> dose. If both immunogenicity tests in paragraph (b) of this section are conducted and a different amount of virus is used in each test, the virus titer requirements shall be based on the higher of the two amounts.

(2) [Reserved]

[60 FR 14361, Mar. 17, 1995, as amended at 72 FR 72564, Dec. 21, 2007]

§ 113.306 Canine Distemper Vaccine.

Canine Distemper Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. 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) *Master Seed Virus.* The Master Seed Virus shall meet the applicable requirements prescribed in §113.300 and the requirements prescribed in this section.

(1) To detect ferret virulent canine distemper virus, each of five canine distemper susceptible ferrets shall be injected with a sample of the Master Seed Virus equivalent to the amount of virus to be used in one dog dose and observed each day for 21 days. If undesirable reactions are observed during the observation period, the lot of Master Seed is unsatisfactory.

(2) Master Seed Virus propagated in tissues or cells of avian origin shall be tested for pathogens by the chicken embryo test prescribed in §113.37. If found unsatisfactory, the Master Seed Virus shall not be used.

(b) 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 canine distemper susceptible dogs shall be used as test animals (20 vaccinates and 5 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 using 50 to 300 TCID<sub>50</sub> of canine distemper 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. The 20 dogs used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five dogs 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 14 days post-injection, the vaccinates and the controls shall each be challenged intracerebrally with virulent canine distemper virus furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 21 days.

(i) If at least four of the five controls do not die and the survivor, if any, does not show clinical signs of canine distemper the test is inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates do not survive without showing clinical signs of canine distemper during the observation period, the Master Seed Virus is unsatisfactory.

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

(c) *Test requirements for release.* Except for §113.300(a)(3)(ii), 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) The test for pathogens prescribed in §113.37 shall be conducted on each serial or one subserial of avian origin vaccine.