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 $10^{0.7}$ greater than that used in such immunogenicity test but not less than $10^{2.5}$ $TCID_{50}$ 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]

§ 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 $TCID_{50}$ 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.

(iii) The Master Seed Virus shall be retested for immunogenicity for canine hepatitis in 3 years unless use of the lot previously tested is discontinued. Ten susceptible dogs (8 vaccinates and 2 controls) shall be used in the retest. Susceptibility shall be determined in

the manner provided in paragraph (b)(1) of this section.

(A) Each vaccinate shall be injected with a predetermined quantity of vaccine virus as provided in paragraph (b)(1)(i) of this section.

(B) At least 14 days postvaccination, a second serum sample shall be drawn from each dog and tested for neutralizing antibody to canine adenovirus in the same manner used to determine susceptibility.

(C) If the two controls have not remained seronegative at 1:2, the test is inconclusive and may be repeated.

(D) If at least six of the eight vaccinates in a valid test do not develop titers of at least 1:10 based upon final serum dilution, the Master Seed Virus is unsatisfactory except as provided in paragraph (b)(1)(iii)(E) of this section.

(E) If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and the controls may be challenged as provided in paragraph (b)(1)(ii) of this section. A Master Seed is satisfactory if all vaccinates remain free of clinical signs of canine hepatitis, while both controls develop severe clinical signs of canine hepatitis. If both controls do not show severe clinical signs of canine hepatitis, the test is inconclusive and may be repeated: *Provided*, That, if any of the vaccinates show such signs, 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 $TCID_{50}$ 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) 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.

(A) If less than 4 of 6 or 2 of 3 of the controls show clinical signs of canine adenovirus type 2 other than fever, the test is inconclusive and may be repeated.

(B) A significant difference in clinical signs shall be demonstrated between vaccinates and controls in a valid test as prescribed in paragraph (b)(2)(ii)(B) of this section.

(iv) 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 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 $10^{2.5}$ $TCID_{50}$ 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]

§ 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 $TCID_{50}$ 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) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Ten susceptible dogs (8 vaccinates and 2 controls) shall be used in the retest. Susceptibility shall be determined in the manner provided in paragraph (b)(1) of this section.

(i) Each vaccinate shall be injected with a predetermined quantity of vaccine virus as provided in paragraph (b)(2) of this section.

(ii) At least 14 days postvaccination, a second serum sample shall be drawn