the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated: *Provided*, That, if the test is not repeated, the serial is unsatisfactory.

(2) *Potency test—serum neutralization test*. Bulk or final container samples of completed product shall be tested for potency using five susceptible dogs (four vaccinates and one control) as the test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine distemper virus to determine susceptibility.

(i) A constant virus-varying serum neutralization test in tissue culture using 50 to 300 $TCID_{50}$ of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(ii) *Vaccination*. Each of the four vaccinates shall be injected as recommended on the label. If two doses are recommended, the second dose shall be administered at the time specified on the label. The dogs shall be observed each day for at least 14 days after the last inoculation.

(iii) *Serology*. At the end of the post vaccination observation period, a second blood sample shall be obtained from each of the five dogs and the serums shall be individually tested for neutralizing antibody against canine distemper virus in the same manner used to determine susceptibility.

(iv) *Interpretation of the serum neutralization test*. If the control has not remained seronegative at 1:2, the test is inconclusive and may be repeated. If at least three of the four vaccinates in a valid test have not developed titers based upon a final serum dilution of at least 1:50 and the remaining vaccinate has not developed a titer of at least 1:25, the serial is unsatisfactory except as provided in paragraphs (c)(2)(v) and (vi) of this section.

(v) *Virus challenge test*. If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and the control may be challenged intracerebrally with a virulent canine distemper virus furnished or approved by the Animal and Plant Health Inspection Service and each animal observed each day for an additional 21 days.

(vi) *Interpretation of the virus challenge test*. For a serial to be satisfactory, all vaccinates must remain free from clinical signs of canine distemper while the control must die of canine distemper. If the control does not die of canine distemper, the test is inconclusive and may be repeated except, that if any of the vaccinates show signs or dies of canine distemper, the serial is unsatisfactory.

[60 FR 14359, Mar. 17, 1995]

### § 113.202 Canine Hepatitis and Canine Adenovirus Type 2 Vaccine, Killed Virus.

Canine Hepatitis and Canine Adenovirus Type 2 Vaccine, Killed Virus, 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 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 requirements prescribed in § 113.200.

(b) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity by one or both of the following methods. Vaccine used for these tests shall be at the highest passage from the Master Seed and prepared at the minimum preinactivation titer specified in the Outline of Production.

(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) The 20 dogs to be used as vaccinates shall be injected with one dose of vaccine and the remaining five dogs held as controls. If a second dose is recommended, the second dose shall be administered at the time specified on the label.

(ii) Not less than 14 days after the last inoculation, each vaccinate and control shall 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.

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

(iv) 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 $TCID_{50}$ of canine adenovirus.

(i) The 20 dogs to be used as vaccinates shall be injected with one dose of vaccine and the remaining 10 dogs held as controls. If a second dose is recommended, the second dose shall be administered at the time specified on the label.

(ii) Not less than 14 days after the last inoculation, 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.

(iii) 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.

(iv) 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.

(c) *Test requirements for release.* Each serial shall meet the applicable general requirements prescribed in § 113.200, the special requirements for safety provided in this section, and the applicable potency tests provided in this section.

(1) *Safety test.* The vaccinates used in the potency test in paragraph (c)(2) and/or (c)(3) of this section shall be observed each day during the postvaccination observation period. If unfavorable reactions occur which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated: *Provided*, That, if not repeated, the serial is unsatisfactory.

(2) *Potency test for canine hepatitis—serum neutralization test.* Bulk or final container samples of completed product shall be tested for potency using at least five susceptible dogs (four vaccinates and one control) as the test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine adenovirus to determine susceptibility.

(i) A constant virus-varying serum neutralization test in tissue culture using 50 to 300 $TCID_{50}$ of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(ii) *Vaccination.* Each of the vaccinates shall be injected as recommended on the label. If two doses are recommended, the second dose shall be administered at the time specified on the label. The dogs shall be observed each day for at least 14 days after the last inoculation.

(iii) *Serology.* At the end of the postvaccination observation period, a second blood sample shall be obtained from each of the dogs and the serums shall be individually tested for neutralizing antibody against canine adenovirus in the same manner used to determine susceptibility.

(iv) *Interpretation of the serum neutralization test.* If the control(s) has not remained seronegative at 1:2, the test is inconclusive and may be repeated. If at

least 75 percent of the vaccinates in a valid test have not developed titers based upon final serum dilution of at least 1:10 and the remaining vaccinate(s) has not developed a titer of at least 1:2, the serial is unsatisfactory except as provided in paragraphs (c)(2)(v) and (vi) of this section.

(v) *Virus challenge test.* If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and the control(s) may be challenged intravenously with a virulent canine hepatitis virus furnished or approved by the Animal and Plant Health Inspection Service and each animal observed each day for an additional 14 days.

(vi) *Interpretation of the virus challenge test.* For a serial to be satisfactory, all vaccinates must remain free of clinical signs of canine hepatitis while the control(s) must show severe clinical signs of canine hepatitis. If the control(s) does not show severe clinical signs of canine hepatitis, the test is inconclusive and may be repeated: *Provided,* That, if any of the vaccinates show signs or die of canine hepatitis, the serial is unsatisfactory.

(3) *Potency test for canine adenovirus type 2.* Bulk or final container samples of completed product shall be tested for potency using eight susceptible dogs (five vaccinates and three controls) as the test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine adenovirus to determine susceptibility.

(i) A constant virus-varying serum neutralization test in tissue culture using 50 to 300 $TCID_{50}$ of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(ii) *Vaccination.* Each of the five vaccinates shall be injected as recommended on the label. If two doses are recommended, the second dose shall be administered at the time specified on the label. The dogs shall be observed each day for at least 14 days after the last inoculation.

(iii) Not less than 14 days after the last inoculation, 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.

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

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

[60 FR 14359, Mar. 17, 1995]

### § 113.203 Feline Panleukopenia Vaccine, Killed Virus.

Feline Panleukopenia Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed. The Master Seed shall meet the applicable requirements prescribed in § 113.200. Each serial shall meet the applicable general requirements prescribed in § 113.200 and the special requirements for safety and potency provided in this section.

(a) *Safety test.* The vaccinates used in the potency test in paragraph (b) of this section shall be observed each day during the postvaccination observation period. If unfavorable reactions occur which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated: *Provided,* That, if not repeated, the serial is unsatisfactory.

(b) *Potency test—serum-neutralization test.* Bulk or final container samples of completed product shall be tested for potency using five susceptible cats (four vaccinates and one control) as the test animals. Blood samples drawn from each cat shall be individually tested for neutralizing antibody