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 vaccines in a valid test have not developed titers based upon a final serum dilution of at least 1:50 and the remaining vaccine 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 vaccines 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 vaccines 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 vaccines 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\textsubscript{50} of canine adenovirus.

(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
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serum dilution in a varying serum-con-
stant virus neutralization test using 50
to 300 TCID₅₀ of canine adenovirus.

(i) The 20 dogs to be used as vacci-
cinates shall be injected with one dose of
vaccine and the remaining 10 dogs held as controls. If a second dose is rec-
commended, the second dose shall be ad-
ministered 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 ex-
posure to a nebulized aerosol of viru-
rent 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 res-
piratory or other clinical signs of ca-
nine adenovirus type 2 noted and re-
corded 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 clin-
cical signs in a valid test cannot be
demonstrated between vaccinates and
controls using a scoring system ap-
proved 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 pro-
vided in this section, and the applica-
ble potency tests provided in this sec-
tion.

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

(2) Potency test for canine hepatitis—
serum neutralization test. Bulk or final
container samples of completed prod-
uct shall be tested for potency using at
least five susceptible dogs (four vacci-
cinates and one control) as the test
animals. Blood samples drawn from
each dog shall be individually tested
for neutralizing antibody against can-
nine adenovirus to determine suscepti-
bility.

(i) A constant virus-varying serum
neutralization test in tissue culture
using 50 to 300 TCID₅₀ of virus shall be
used. Dogs shall be considered suscept-
tible if there is no neutralization at a
1:2 final serum dilution.

(ii) Vaccination. Each of the vacci-
cinates shall be injected as rec-
ommended on the label. If two doses
are recommended, the second dose
shall be administered at the time spec-
ified on the label. The dogs shall be ob-
served 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 neutral-
izing antibody against canine
adenovirus in the same manner used to
determine susceptibility.

(iv) Interpretation of the serum neutral-
ization test. If the control(s) has not re-
mained 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 vac-
cinate(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 intra-
venously with a virulent canine hepa-
titis 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 chal-
lenge test. For a serial to be satisfac-
tory, 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
Animal and Plant Health Inspection Service, USDA

§ 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 against feline panleukopenia virus to determine susceptibility.

(1) A constant virus-varying serum neutralization test in tissue culture using 100 to 300 TCID50 of virus shall be used. Cats shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(2) Vaccination. Each of the four vaccinates shall be injected as recommended on the label. If two doses are recommended, the second dose shall be given 7 to 10 days after the first dose and the cats observed each day for 14 to 21 days.

(3) Serology. At the end of the postvaccination observation period, a second blood sample shall be obtained from each of the five cats and the serums shall be individually tested for neutralizing antibody against feline panleukopenia virus to determine susceptibility.