within the expiration period, each se-
rial and subserial shall have a virus
titer equal to or greater than that used
in the immunogenicity test.

(3) Young adult mice, each weighing
14 to 16 grams, shall be used as test ani-
mals when the virus in vaccine pre-
pared with a low egg passage Flury
Strain or high cell passage Street Ala-
bama DeBoto Strain (HCP SAD) of ra-
bies virus is titrated. At least 10 mice
for each dilution shall be used.

(i) At least 10 mice shall be used for
each dilution. Each shall be injected
intracerebrally with 0.03 ml.

(ii) The injected young adult mice
shall be observed each day for 14 days
except when testing vaccines made
with HCP SAD strain of rabies virus, in
which case, the mice shall be observed
each day for 21 days. Deaths and paral-
ysis occurring subsequent to the fourth
day post-injection shall be noted and
the LD_{50} titer calculated by the Reed
and Muench Method.

(iii) Virus titer requirements for re-
lease and at expiration date shall be
determined for each vaccine on the
basis of data available: Provided, That,
the lowest titer permitted at expira-
tion date when determined by this test
shall be 10^{3.0} LD_{50} per 0.02 ml.

(4) Suckling mice, 6 days of age or
younger, shall be used as test animals
when virus in vaccine prepared with a
high egg passage Flury Strain of rabies
virus is titrated.

(i) Six to twelve mice shall be used
for each dilution. Each shall be in-
jected intracerebrally with 0.02 ml.

(ii) The injected suckling mice shall
be observed each day for 21 days.
Deaths and paralysis occurring subse-
quent to the fourth day post-injection
shall be noted and the LD_{50} titer cal-
culated by the Reed and Muench Meth-
od; and

(iii) Virus titer requirements for re-
lease and at expiration date shall be
determined for each vaccine on the
basis of data available: Provided, That,
the lowest titer permitted at expira-

§ 113.313 Measles Vaccine.

Measles 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 pre-
paring the production seed virus for
vaccine production. All serials of vac-
cine 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. Each lot of Mas-
ter Seed Virus shall meet the special
requirements prescribed in this sec-
tion.

(b) To detect virulent canine dis-
temper virus, each of two canine dis-
temper susceptible ferrets shall be in-
jected with a sample of the Master
Seed Virus equivalent to the amount of
virus to be used in one dog dose and ob-
served each day for 21 days. If undesir-
able reactions occur in either ferret,
the lot of Master Seed Virus is unsatis-
factory.

(c) Each lot of Master Seed Virus
used for vaccine production shall be
tested for immunogenicity. The se-
lected virus dose from the lot of Master
Seed Virus shall be established as fol-
low:

(1) Twenty-five dogs, less than 12
weeks of age and free of measles anti-
body, shall be used as test animals (20
vaccinates and five controls). Blood
samples shall be drawn from these ani-
mals 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 vary-
ing serum-constant virus neutraliza-
tion test with less than 500 ID_{50}
of mea-
sles virus.

(2) A geometric mean titer of the
dried vaccine produced from the high-
est 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) 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 10^2.7 greater than that used in the immunogenicity test but not less than 10^2.5 ID50 per dose.

§ 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 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