(ii) A test for safety in three young seronegative animals of the most susceptible species for which the vaccine is recommended shall be conducted. Each shall be injected intramuscularly with 10 recommended doses of vaccine. If unfavorable reactions attributable to the product occur during a 28-day observation period, the serial is unsatisfactory.

(iii) If primary cell cultures of hamster origin or of mouse origin are used vaccine production, they shall be tested for LCM virus as prescribed in §113.42. The cells shall be disrupted and undiluted cell fluids from each lot shall be tested.

(2) **Virus titrations.** Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (b)(1) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently higher than the titer of the vaccine virus used 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 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 animals when the virus in vaccine prepared with a low egg passage Flury Strain or high cell passage Street Alabama Dufferin Strain (HCP SAD) of rabies 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 paralysis 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 release and at expiration date shall be determined for each vaccine on the basis of data available: Provided, That, the lowest titer permitted at expiration date when determined by this test shall be $10^{3.0}$ LD$_{50}$ per 0.03 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 injected intracerebrally with 0.02 ml.

(ii) The injected suckling mice shall be observed each day for 21 days. Deaths and paralysis occurring subsequent to the fourth day post-injection shall be noted and the LD$_{50}$ titer calculated by the Reed and Muench Method; and

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

§ 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 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. Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(b) To detect virulent canine distemper virus, each of two 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 occur in either ferret, the lot of Master Seed Virus is unsatisfactory.

(c) Each lot of Master Seed Virus used for vaccine production shall be
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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.