

§ 113.328

9 CFR Ch. I (1-1-10 Edition)

by the eye-drop method with the equivalent of 10 doses of vaccine and observed each day for 21 days post-vaccination. Severe respiratory signs or death shall be counted as failures. Two-stage sequential testing may be conducted if the first test (which then becomes stage one) has three failures.

(ii) The results shall be evaluated according to the following table:

CUMULATIVE TOTALS			
Stage	Number of chickens	Failures for satisfactory serials	Failures for unsatisfactory serials
1	25	2 or less	4 or more.
2	50	5 or less	6 or more.

If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated or, in lieu thereof, the serial declared unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the procedure prescribed in paragraph (c)(2) of this section and in this paragraph.

(i) The Newcastle disease virus fraction of combined Newcastle-Bronchitis Vaccines shall be neutralized prior to titration of the bronchitis virus fraction. Equal parts of heat-inactivated Newcastle disease antiserum shall be mixed with each appropriate serial ten-fold dilution of the vaccine. After inactivation, embryos shall be injected with 0.2 ml each and results calculated as a 0.1 ml dose to allow for serum dilution of the vaccine. The allantoic fluids, tested as prescribed in §113.34 shall not show hemagglutinating activity in the lowest dilution used in the titration.

(ii) Each bronchitis virus type shall be harvested separately and a sample of bulk harvested material shall be collected prior to mixing with the other virus type(s). Each sample shall contain not less than the minimum virus titer stated in the filed Outline of Production.

(iii) 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 $10^{9.7}$ greater than that used in such immunogenicity test but not less than $10^{2.0}$ EID₅₀ per dose.

[39 FR 44724, Dec. 27, 1974, as amended at 40 FR 18406, Apr. 28, 1975; 40 FR 41089, Sept. 5, 1975; 42 FR 43617, Aug. 30, 1977; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 64 FR 43045, Aug. 9, 1999; 72 FR 72564, Dec. 21, 2007]

§ 113.328 Fowl Laryngotracheitis Vaccine.

Fowl Laryngotracheitis 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 in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for 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 and the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of vaccine virus override, the test may be repeated and if the repeat test is inconclusive for the same reason, the chicken inoculation test prescribed in §113.36 may be conducted and the virus judged accordingly. Each lot shall also be tested for safety as follows:

(1) Each of at least ten 3 to 4 week old susceptible chickens obtained from the same source and hatch as those used in the immunogenicity test prescribed in paragraph (c) of this section shall be injected intratracheally with 0.2 ml of the virus as used in the vaccine and the chickens observed each day for 14 days.

(2) If more than 20 percent of the chickens die during the observation period, the virus is unsatisfactory.

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

tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Fowl laryngotracheitis susceptible chickens all of the same age and from the same source shall be used. Twenty or more chickens shall be used as vaccinates for each method of administration recommended on the label. Ten additional chickens of the same age and from the same source shall be held as unvaccinated controls.

(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. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each chicken used in the test. At least three appropriate (not to exceed tenfold) dilutions shall be used for vaccine of chicken embryo origin and the test conducted as follows:

(i) For each dilution, inject at least five embryos, 9 to 11 days old, on the chorioallantoic membrane with 0.2 ml each. Disregard all deaths during the first 24 hours post-injection. To be a valid test, at least four embryos in each dilution shall remain viable beyond 24 hours.

(ii) Examine the surviving embryos for evidence of infection 5 to 8 days post-injection.

(iii) A satisfactory titration shall have at least one dilution with between 50 and 100 percent positives and at least one dilution with between 50 and 0 percent positives.

(iv) Calculate the EID_{50} by the Spearman-Kärber or Reed-Muench method.

(3) Tissue culture origin vaccine may be titrated by a tissue culture method approved by Animal and Plant Health Inspection Service and written into the filed Outline of Productions.

(4) Ten to fourteen days post-vaccination, all vaccinates and controls shall be challenged intratracheally or in the orbital sinus with infectious fowl laryngotracheitis virus and observed each day for 10 days. Challenge virus shall be provided or approved by Ani-

mal and Plant Health Inspection Service.

(5) If at least 80 percent of the controls do not die or show clinical signs of fowl laryngotracheitis during the observation period, the test is inconclusive and may be repeated. If at least 19 of 20, 27 of 30, or 36 of 40 of the vaccinates in each group do not remain free of clinical signs of fowl laryngotracheitis during the observation period, the Master Seed Virus is unsatisfactory.

(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) After a lot of Master Seed Virus has been established as prescribed in paragraphs (a), (b), and (c) of this section, each serial and subserial shall meet the applicable requirements in §113.300 and the requirements prescribed in this paragraph.

(1) Final container samples from each serial shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the chicken inoculation test prescribed in §113.36 may be conducted and the vaccine judged accordingly.

(2) *Safety test.* Final container samples of completed product from each serial of modified live virus vaccine shall be tested for safety as provided in this paragraph. Live virus vaccine not prepared with modified live virus shall be tested for safety as provided in the filed Outline of Production.

(i) Twenty-five 3 to 4 week old laryngotracheitis susceptible chickens shall be injected intratracheally with 0.2 ml of vaccine rehydrated at the rate of 30 ml for 1,000 doses. Chickens shall be observed each day for 14 days. Deaths shall be counted as failures. Two-stage sequential testing may be conducted if the first test (which then becomes stage one) has five, six, or seven failures.

(ii) The results shall be evaluated according to the following table:

CUMULATIVE TOTALS

Stage	Number of chickens	Failures for satisfactory serials	Failures for unsatisfactory serials
1	25	4 or less	8 or more.
2	50	10 or less	11 or more.

(iii) If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and repeated or in lieu thereof, the serial declared unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method provided in paragraphs (c)(2) or (3) 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 $10^{2.5}$ EID₅₀ per dose for chicken embryo origin vaccine and $10^{2.0}$ EID₅₀ or $10^{2.5}$ TCID₅₀ per dose for tissue culture origin vaccine.

[39 FR 44726, Dec. 27, 1974, as amended at 40 FR 18407, Apr. 28, 1975; 40 FR 41089, Sept. 5, 1975; 41 FR 44359, Oct. 8, 1976; 42 FR 43617, Aug. 30, 1977; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 72 FR 72564, Dec. 21, 2007]

§ 113.329 Newcastle Disease Vaccine.

Newcastle Disease 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 in accordance with the requirements in paragraphs (a), (b), and (c) of this section shall be used for 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 §113.34, and the requirements prescribed in this section.

(b) Each lot of Master Seed Virus shall be tested for pathogens by the chicken embryo inoculation test prescribed in §113.37, except that, if the test is inconclusive because of a vaccine virus override, the test may be repeated and if the repeat test is inconclusive for the same reason, the chicken inoculation test prescribed in §113.36 may be conducted and the virus judged accordingly.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity and the selected virus dose to be used shall be established as follows:

(1) Newcastle Disease susceptible chickens, all of the same age and from the same source, shall be used. Twenty or more chickens shall be used as vaccinates for each method of administration recommended on the label. Ten additional chickens of the same age and from the same source shall be held as unvaccinated controls.

(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. Each vaccinate shall receive a predetermined quantity of vaccine virus. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the amount of virus administered to each chicken used in the test. At least three appropriate (not to exceed tenfold) dilutions shall be used and the test conducted as follows:

(i) For each dilution, inject at least five embryos, 9 to 11 days old, in the allantoic cavity with at least 0.1 ml each. Disregard all deaths during the first 24 hours post-injection. To be a valid test, at least four embryos in each dilution shall remain viable beyond 24 hours.

(ii) Examine the surviving embryos for evidence of infection 5 to 7 days post-injection.

(iii) A satisfactory titration shall have at least one dilution with between 50 and 100 percent positives and at least one dilution with between 50 and 0 percent positives.

(iv) Calculate the EID₅₀ by the Spearman-Kärber or Reed-Muench method.