pox during the observation period, the Master Seed Virus is unsatisfactory.

(5) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and the controls shall meet the criteria prescribed in paragraph (c)(4) of this section.

(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 requirements in § 113.36, in § 113.300 except paragraph (c), and in this paragraph.

(1) *Safety test.* Final container samples of completed product from each serial shall be tested. Vaccines recommended for use in birds 10 days of age or younger shall be tested in accordance with paragraphs (d)(1)(i), (ii), and (iii) of this section.

(i) Each of 25 susceptible birds 5 days of age or younger, properly identified and obtained from the same source and hatch, shall be vaccinated with the equivalent of 10 doses of vaccine by each of all routes recommended on the label and observed each day for 14 days. Severe clinical 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 birds	Failures for satisfactory serials	Failures for unsatisfactory serials
1	25	2 or less	4 or more.
2	50	5 or less	6 or more.

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

(iv) Vaccines not recommended for use in birds 10 days of age or younger shall be tested for safety as follows: Each of twenty-five 3- to 5-week-old, fowl-pox susceptible birds shall be vaccinated with the equivalent of 10 doses of vaccine by each of all routes recommended on the label and observed each day for 14 days. If any of the birds show severe clinical signs of disease or death during the observation period due to causes attributable to the product, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the product, the test shall be declared inconclusive and may be repeated or, in lieu thereof, the serial declared unsatisfactory.

(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 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 $10^{2.0}$ EID_{50} per dose.

[39 FR 44724, Dec. 27, 1974, as amended at 40 FR 18406, Apr. 28, 1975; 40 FR 41089, Sept. 5, 1975; 44 FR 33051, June 8, 1979; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.327 Bronchitis Vaccine.

Bronchitis 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 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) Bronchitis susceptible chickens, all of the same age and from the same source, shall be used in the virus-recovery test. For each method of administration recommended on the label for each serotype against which protection is claimed, twenty or more chickens shall be used as vaccinates. Ten additional chickens for each serotype against which protection is claimed 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 tests are 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 such tests. At least three approved (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 0.1 ml each. Deaths occurring during the first 24 hours shall be disregarded, but at least four viable embyros in each dilution shall survive beyond 24 hours of a valid test. After 5 to 8 days incubation, examine the surviving embryos for evidence of infection.

(ii) 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.

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

(3) Twenty-one to twenty-eight days post-vaccination, all vaccinates and controls shall be challenged by eyedrop with virulent bronchitis virus. A separate set of vaccinates and controls shall be used for each serotype against which protection is claimed. Each challenge virus shall be approved or provided by Animal and Plant Health Inspection Service and shall titer at least $10^{4.0}$ EID_{50} per ml.

(i) Tracheal swabs shall be taken once, 5 days post-challenge, from each control and vaccinate. Each swab shall be placed in a test tube containing 3 ml of tryptose phosphate broth and antibiotics. The tube and swab shall be swirled thoroughly and if they are to be stored, be immediately frozen and be stored at below −40 °C. pending egg evaluation. For each chicken swab, at least five chicken embryos 9 to 11 days old shall be inoculated in the allantoic cavity with 0.2 ml each of broth from each tube.

(ii) All embryos surviving the third day post-inoculation shall be used in the evaluation, except that, if a swab is not represented by at least four embryos, the test of that swab is invalid and the results inconclusive. A tracheal swab shall be positive for virus recovery when any of the embryos in a valid test show typical infectious bronchitis virus lesions, such as but not limited to, stunting, curling, kidney urates, clubbed down, or death during the 4 to 7 day post-inoculation period. If less than 20 percent of the embryos which survive the third day post-inoculation die during the 4 to 7 day post-inoculation period and show no gross lesions typical of infectious bronchitis, they may be disregarded.

(iii) If less than 90 percent of the controls are positive for virus recovery, the test is inconclusive and may be repeated.

(iv) If less than 90 percent of the vaccinates are negative for virus recovery, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only one method of administration recommended on the label need be used in the retest. The vaccinates and the controls shall meet the

criteria prescribed in paragraph (c)(3) of this section.

(5) 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, except that, if the vaccine contains more than one virus type, bulk samples taken from each type prior to mixing shall be used in the virus identity tests prescribed in § 113.300(c). The additional requirements in this paragraph shall also be met.

(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 shall be tested to determine safety for use in bronchitis susceptible young chickens.

(i) Twenty-five susceptible chickens, 5 days of age or younger, properly identified and obtained from the same source and hatch, shall be vaccinated 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 tenfold 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 $10^{0.7}$ greater than that used in such immunogenicity test but not less than $10^{2.0}$ EID_{50} 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]

§ 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