frozen vaccine shall be reconstituted in diluent according to the label recommendations, and held in an ice bath at 0 °C to 4 °C for 2 hours prior to use in the potency test.

(ii) For a serial or subserial to be eligible for release, each serotype contained in the vaccine shall have a virus titer per dose which is at least 3 times greater than the number of plaque forming units (pfu) used in the immunogenicity test prescribed in paragraph (c) of this section, but not less than 1000 pfu per dose.

(iii) When tested (without the pretest incubation of desiccated products) at any time within the expiration period, each serotype contained in the vaccine shall have a virus titer per dose which is at least 2 times the number of pfu used in the immunogenicity test, but not less than 750 pfu per dose.

§ 113.331 Bursal Disease Vaccine.

Bursal 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 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 chicken inoculation test prescribed in §113.36 may be conducted and the virus judged accordingly. Each lot of Master Seed Virus used in the preparation of modified live virus vaccines shall also be nonpathogenic to chickens as determined by the following procedures:

(i) Each of twenty-five 1-day-old bursal disease susceptible chickens (vaccinates) shall be injected subcutaneously with 10 times the recommended dose of vaccine virus and observed for 21 days. Fifteen chickens of the same source and hatch shall be kept isolated as controls.

(ii) Seventeen days postvaccination, each of five controls shall be administered at least $10^{2.0}$ EID$_{50}$ of a virulent bursal disease virus by eye-drop, isolated, and used as positive controls. The remaining controls shall be used as negative controls.

(iii) If the vaccinates do not remain free of clinical signs of bursal disease, the Master Seed Virus is unsatisfactory. If unfavorable reactions which are not attributable to the Master Seed Virus occur in more than two of the vaccinates, the test shall be declared inconclusive and may be repeated.

(2) Each of thirty-five 3- to 4-week-old bursal disease susceptible chickens (vaccinates) shall be vaccinated with approximately one minimum protective dose of vaccine virus as determined in paragraph (c) of this section. Each of 10 chickens of the same source and hatch shall be administered at least $10^{2.0}$ EID$_{50}$ of a virulent bursal disease virus by eye-drop, isolated, and used as positive controls. Also, each of 20 additional chickens of the same source and hatch shall be isolated and held as negative controls.

(i) Three or four days postvaccination, 10 of the vaccinates, 10 positive controls, and 10 of the negative controls shall be necropsied and examined for gross lesions of bursal disease. If any of the negative controls or less than 8 of the positive controls have such lesions, the test is inconclusive.
and may be repeated. For purposes of this test, gross lesions shall include peri-bursal edema and/or edema and/or macroscopic hemorrhage in the bursal tissue.

(ii) Fourteen days post-vaccination, the remaining vaccinates and negative controls shall be necropsied and examined for obvious bursal atrophy. If any of the vaccinates have such atrophy, the Master Seed Virus is unsatisfactory, except that, if any of the negative controls have such atrophy, the test is inconclusive and may be repeated.

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

(1) Bursal Disease susceptible chickens, all of the same age (3 weeks or younger) 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 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 ten-fold) dilutions shall be used to conduct the titrations by a method acceptable to Animal and Plant Health Inspection Service.

(3) When the test chickens are 28 to 35 days of age but not less than 14 days postvaccination, each vaccinate and each control shall be challenged by eye-drop with a virulent bursal disease virus provided or approved by Animal and Plant Health Inspection Service.

(i) Three to five days postchallenge, all vaccinates and controls shall be necropsied and examined for gross lesions of bursal disease as described in paragraph (b)(2)(i) of this section.

(ii) If at least 19 of 20, or 27 of 30, or 36 of 40 vaccinates in each group are not free from such lesions, the Master Seed Virus is unsatisfactory, except that, if less than 90 percent of the controls have such lesions, the test is inconclusive and may be repeated.

(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) Tests for pathogens. 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 over-ride, the chicken inoculation test prescribed in §113.36 may be conducted and the serial judged accordingly.

(2) Safety tests. (i) Final container samples of completed product from each serial shall be tested to determine whether the vaccine is safe as follows:

(A) For vaccines intended for parenteral administration, each of twenty-five 1-day-old bursal disease susceptible chickens shall be vaccinated with the equivalent of 10 doses by subcutaneous injection.

(B) For vaccines intended for drinking water administration, each of twenty-five 4- to 5-week-old bursal disease susceptible chickens shall be vaccinated orally with the equivalent of 10 doses.

(C) Ten chickens of the same source and hatch shall be maintained in isolation as negative controls. The vaccinates and controls shall be observed each day for 21 days.

(ii) If unfavorable reactions which are attributable to the biological product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur in more than one of the controls or if unfavorable reactions which are not attributable to the biological product occur in more than two of the vaccinates, the test shall be declared inconclusive and repeated, except that, if the test is not repeated, the serial shall be unsatisfactory.
(3) 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} times greater than that used in such immunogenicity test, but not less than 10^{2.0} titration units (PFU or ID_{50}'s) per dose.

(4) Safety using the following chicken test:

(i) For vaccines intended for use in chickens less than 14 days of age, Master Seed equal to 10 label doses shall be administered subcutaneously to each of 25 1-day-old tenosynovitis susceptible chickens.

(ii) For vaccines intended for use only in chickens 14 days of age or older, Master Seed equal to 10 label doses shall be administered subcutaneously to each of 25 4-week-old or older tenosynovitis susceptible chickens.

(iii) The vaccinates shall be observed each day for 21 days. If unfavorable reactions occur which are attributable to