§ 113.330 Marek’s Disease Vaccines.

Marek’s disease vaccine shall be prepared from virus-bearing tissue culture cells. 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.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in §113.300, and the requirements prescribed in this section. The identity test required in §113.300(c) shall be conducted in a serotype-specific manner by a method acceptable to APHIS. Each lot of Master Seed Virus shall also 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.

(b) Safety test. The Master Seed Virus shall be nonpathogenic for chickens as determined by the following procedure:

(i) Specific pathogen free chickens or embryos, negative for Marek’s disease virus antibodies, and from the same source, shall be isolated into the following groups:

(ii) Group 1. At least 50 test subjects shall be inoculated with 10 times as much viable virus as will be contained in one dose of vaccine, by the route recommended for vaccination.

(ii) Group 2. At least 50 test subjects shall be injected with a very virulent Marek’s disease virus provided or approved by APHIS, at a dosage level that will cause gross lesions of Marek’s disease in at least 80 per cent of the chickens within 50 days.

(iii) Group 3. Fifty uninoculated controls. For in ovo studies, this group should receive a sham inoculation of diluent.

(iv) Group 4. For studies evaluating Serotype 1 Master Seed Viruses, a group of 50 uninoculated control chickens shall be housed in contact with the group 1 vaccinated chickens.

(b) Safety test. The Master Seed Virus shall be nonpathogenic for chickens as determined by the following procedure:

(i) Specific pathogen free chickens or embryos, negative for Marek’s disease virus antibodies, and from the same source, shall be isolated into the following groups:

(ii) Group 1. At least 50 test subjects shall be inoculated with 10 times as much viable virus as will be contained in one dose of vaccine, by the route recommended for vaccination.

(ii) Group 2. At least 50 test subjects shall be injected with a very virulent Marek’s disease virus provided or approved by APHIS, at a dosage level that will cause gross lesions of Marek’s disease in at least 80 per cent of the chickens within 50 days.

(iii) Group 3. Fifty uninoculated controls. For in ovo studies, this group should receive a sham inoculation of diluent.

(iv) Group 4. For studies evaluating Serotype 1 Master Seed Viruses, a group of 50 uninoculated control chickens shall be housed in contact with the group 1 vaccinated chickens.

(b) Safety test. The Master Seed Virus shall be nonpathogenic for chickens as determined by the following procedure:

(i) Specific pathogen free chickens or embryos, negative for Marek’s disease virus antibodies, and from the same source, shall be isolated into the following groups:

(ii) Group 1. At least 50 test subjects shall be inoculated with 10 times as much viable virus as will be contained in one dose of vaccine, by the route recommended for vaccination.

(ii) Group 2. At least 50 test subjects shall be injected with a very virulent Marek’s disease virus provided or approved by APHIS, at a dosage level that will cause gross lesions of Marek’s disease in at least 80 per cent of the chickens within 50 days.

(iii) Group 3. Fifty uninoculated controls. For in ovo studies, this group should receive a sham inoculation of diluent.

(iv) Group 4. For studies evaluating Serotype 1 Master Seed Viruses, a group of 50 uninoculated control chickens shall be housed in contact with the group 1 vaccinated chickens.

(b) Safety test. The Master Seed Virus shall be nonpathogenic for chickens as determined by the following procedure:

(i) Specific pathogen free chickens or embryos, negative for Marek’s disease virus antibodies, and from the same source, shall be isolated into the following groups:

(ii) Group 1. At least 50 test subjects shall be inoculated with 10 times as much viable virus as will be contained in one dose of vaccine, by the route recommended for vaccination.

(ii) Group 2. At least 50 test subjects shall be injected with a very virulent Marek’s disease virus provided or approved by APHIS, at a dosage level that will cause gross lesions of Marek’s disease in at least 80 per cent of the chickens within 50 days.

(iii) Group 3. Fifty uninoculated controls. For in ovo studies, this group should receive a sham inoculation of diluent.

(iv) Group 4. For studies evaluating Serotype 1 Master Seed Viruses, a group of 50 uninoculated control chickens shall be housed in contact with the group 1 vaccinated chickens.

(b) Safety test. The Master Seed Virus shall be nonpathogenic for chickens as determined by the following procedure:

(i) Specific pathogen free chickens or embryos, negative for Marek’s disease virus antibodies, and from the same source, shall be isolated into the following groups:

(ii) Group 1. At least 50 test subjects shall be inoculated with 10 times as much viable virus as will be contained in one dose of vaccine, by the route recommended for vaccination.

(ii) Group 2. At least 50 test subjects shall be injected with a very virulent Marek’s disease virus provided or approved by APHIS, at a dosage level that will cause gross lesions of Marek’s disease in at least 80 per cent of the chickens within 50 days.

(iii) Group 3. Fifty uninoculated controls. For in ovo studies, this group should receive a sham inoculation of diluent.

(iv) Group 4. For studies evaluating Serotype 1 Master Seed Viruses, a group of 50 uninoculated control chickens shall be housed in contact with the group 1 vaccinated chickens.
(ii) Group 2. A minimum of 35 nonvaccinated test subjects shall be held as challenge controls.

(iii) Group 3. A minimum of 25 nonvaccinated test subjects shall be held as nonchallenge controls.

(iv) Group 4. Except for studies evaluating vaccines which contain only a Serotype 3 virus as the Marek’s disease fraction, a minimum of 35 chicks shall be vaccinated at 1 day of age with a licensed Serotype 3 vaccine, in order to document the severity of the very virulent challenge.

(2) At least 30 chickens in groups 1, 2, and 4, and at least 20 chickens in group 3, shall survive to 5 days of age. All chickens in groups 1, 2, and 4 shall be challenged at 5 days of age in the following manner:

(i) For studies evaluating vaccines which contain only a Serotype 3 virus as the Marek’s disease fraction, groups 1 and 2 shall be inoculated with a standard virulent challenge virus provided or approved by APHIS.

(ii) For all other Marek’s disease vaccines, groups 1, 2, and 4 shall be inoculated with a very virulent challenge virus provided or approved by APHIS.

(3) All chickens shall be observed until 7 weeks of age, necropsied, and examined for grossly observable lesions consistent with Marek’s disease. Any chickens not so examined shall be scored as positive for Marek’s disease.

(4) For a valid test, at least 80 percent of the chickens in group 2 must develop grossly observable lesions, none of the chickens in group 3 shall develop grossly observable lesions, and (when included) greater than 20 percent of the chickens in group 4 must develop grossly observable lesions.

(5) For a valid test to be considered satisfactory, at least 80 percent of the chickens in group 1 must remain free of grossly observable lesions. The appropriate product claim resulting from a satisfactory test would be to aid in the prevention of very virulent Marek’s disease, for all other vaccines.

(d) Test requirements for release. Each serial and subserial shall meet the applicable requirements prescribed in §113.300. The identity test required in §113.300(c) shall be conducted in a serotype-specific manner by a method acceptable to APHIS. Final container samples of completed product shall also meet the requirements in paragraphs (d) (1), (2), and (3) of this section. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) Purity test. The chicken embryo inoculation test prescribed in §113.37 shall be conducted, 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.

(2) Safety test. At least 25 one-day-old, specific pathogen free chickens shall be injected, by the subcutaneous route, with the equivalent of 10 chicken doses of virus (vaccine concentrated 10X). The chickens shall be observed each day for 21 days. Chickens dying during the period shall be examined, cause of death determined, and the results recorded.

(i) If at least 20 chickens do not survive the observation period, the test is inconclusive.

(ii) If lesions of any disease or cause of death are directly attributable to the vaccine, the serial is unsatisfactory.

(iii) If less than 20 chicks survive the observation period and there are no deaths or lesions attributable to the vaccine, the test may be repeated one time, Provided, that if the test is not repeated, the serial shall be declared unsatisfactory.

(3) Potency test. The samples shall be titrated using a cell culture system or other titration method acceptable to APHIS. For vaccines composed of more than one Marek’s disease virus serotype, each fraction shall be titrated in a serotype-specific manner.

(i) Samples of desiccated vaccine shall be incubated at 37°C for 3 days before preparation for use in the potency test. Samples of desiccated or frozen
§ 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 $10^{5.0}$ 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.

(b) 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) Three or four days postvaccination, 10 of the vaccinates, the 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 have such lesions, the test is conclusive.

(ii) Twenty-one days postvaccination, the vaccinates and the controls shall be necropsied and examined for gross lesions of bursal disease. If any of the negative controls have such lesions, the test is inconclusive.