§ 113.216 Bovine Rhinotracheitis Vaccine, Killed Virus.

Infectious Bovine Rhinotracheitis Vaccine, Killed Virus, 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 seed cultures for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.200 and the requirements of this section.

(b) The immunogenicity of vaccine prepared in accordance with the Outline of Production shall be established by a method acceptable to the Animal and Plant Health Inspection Service. Vaccine used for this test shall be at the highest passage from the Master Seed and at the minimum preinactivation titer provided in the Outline of Production.

(c) *Test requirements for release*. Each serial and subserial shall meet the requirements prescribed in § 113.200 and the special requirements provided in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety*. Vaccinates used in the potency test in paragraph (c)(2) of this section shall be observed each day during the prechallenge period. If unfavorable reactions occur, which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated one time. If the results of the second test are not satisfactory, or if the test is not repeated, the serial is unsatisfactory.

(2) *Potency*. Bulk or final container samples of completed product shall be tested for potency using the method described in this paragraph.

(i) Eight infectious bovine rhinotracheitis susceptible calves (five vaccinates, three controls) shall be used as test animals. Individual serum samples shall be collected, inactivated, and individually tested for neutralizing antibody.

(ii) A constant virus decreasing serum neutralization test in cell culture using 50–300 TCID50 of virus shall be used. Calves shall be considered susceptible if there is no neutralization at 1:2 final serum dilution. Other tests of equal sensitivity acceptable to the Animal and Plant Health Inspection Service may be used.

(iii) The five calves used as vaccinates shall be administered one dose of vaccine as recommended on the label. If two doses are recommended, the second dose shall be given according to the interval recommended on the label.

(iv) Fourteen or more days after the last vaccination, blood samples shall be drawn and the individual serum samples inactivated and tested for infectious bovine rhinotracheitis virus neutralizing antibody by the same method used to determine susceptibility.

(v) *Test interpretation*. If the three controls have not remained seronegative at 1:2, the test is a No Test (NT) and may be repeated. If at least four of the five vaccinates in a valid test have not developed 50 percent endpoint titers of 1:8, the serial is unsatisfactory, except as provided in paragraph (c)(2)(vi) of this section.

(vi) *Virus Challenge Test*. If the results of a valid serum neutralization test are unsatisfactory, the vaccinates and controls may be challenged with virulent infectious bovine rhinotracheitis virus furnished or approved by the Animal and Plant Health Inspection Service. The animals shall be observed each day for 14 days post-challenge. If two of the three control calves do not show a temperature rise to 104.5 °F and develop respiratory or other clinical signs of infectious bovine rhinotracheitis, the test is a No Test (NT) and may be repeated one time. If more than one of the vaccinates shows a temperature of 104.0°F for 2 or more days or if more than one of the vaccinates develops respiratory or clinical or other signs, the serial is unsatisfactory.

(vii) The prevaccination and postvaccination sera from a satisfactory potency test shall be submitted to the National Veterinary Services Laboratories for testing by the Animal and Plant Health Inspection Service.

[55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

LIVE VIRUS VACCINES

§ 113.300 General requirements for live virus vaccines.

When prescribed in an applicable Standard Requirement or in the filed Outline of Production, a live virus vaccine shall meet the applicable requirements in this section.

(a) *Purity tests.* (1) *Bacteria and fungi.* Final container samples of completed product and comparable samples of each lot of Master Seed Virus shall be tested for bacteria and fungi in accordance with the test provided in § 113.27.

(2) *Mycoplasma.* Final container samples of completed product and comparable samples of each lot of Master Seed Virus shall be tested for mycoplasma in accordance with the test provided in § 113.28.

(3) *Avian Origin Vaccine.* Samples of each lot of Master Seed Virus and bulk pooled material or final container samples from each serial shall also be tested for:

(i) Salmonella contamination as prescribed in § 113.30; and

(ii) Lymphoid leukosis virus contamination as prescribed in § 113.31; and

(iii) Hemagglutinating viruses as prescribed in § 113.34.

(4) *Extraneous viruses.* Each lot of Master Seed Virus used to prepare live virus vaccine recommended for animals other than poultry shall meet the requirements for extraneous viruses as prescribed in § 113.55

(b) *Safety tests.* Samples of each lot of Master Seed Virus and final container samples of completed product from each serial or first subserial of live virus vaccine recommended for animals other than poultry shall be tested for safety in at least one species for which the vaccine is intended using methods prescribed in §§ 113.39, 113.40, 113.41, 113.44, and 113.45 or in a filed Outline of Production. The mouse safety test prescribed in § 113.33(a) shall also be conducted unless the virus or agent in the vaccine is inherently lethal for mice.

(c) *Virus identity test.* At least one of the virus identity tests provided in this paragraph or a suitable identity test prescribed in the filed Outline of Production shall be conducted on the Master Seed Virus and final container samples from each serial or first subserial of biological product.

(1) *Fluorescent antibody test.* The fluorescent antibody test shall be conducted using virus inoculated cells and uninoculated control cells. Cells shall be stained with fluorochrome conjugated specific antiserum. Fluorescence typical of the virus concerned shall be demonstrated in the inoculated cells. The control cells shall remain free of such fluorescence.

(2) *Serum neutralization test.* The serum neutralization test shall be conducted using the constant serum-decreasing virus method with specific antiserum. For positive identification, at least 100 ID_{50} of vaccine virus shall be neutralized by the antiserum.

(d) *Cell Culture Requirements.* If cell cultures are used in the preparation of Master Seed Virus or of the vaccine, primary cells shall meet the requirements prescribed in § 113.51, cell lines shall meet the requirements prescribed in § 113.52, and ingredients of animal origin shall meet the applicable requirements in § 113.53.

(e) *Moisture content.* The maximum moisture content in desiccated vaccines shall be stated in the filed Outline of Production.

[39 FR 27430, July 29, 1974, as amended at 43 FR 49528, Oct. 24, 1978; 50 FR 1042, Jan. 9, 1985; 54 FR 19352, May 5, 1989. Redesignated at 55 FR 35562, Aug. 31, 1990; 60 FR 24549, May 9, 1995]

§ 113.301 Ovine Ecthyma Vaccine.

Ovine Ecthyma Vaccine shall be prepared from tissue culture fluids or virus-bearing tissues obtained from sheep that have developed ovine ecthyma following inoculation with virulent ovine ecthyma virus. Ovine Ecthyma Vaccine is exempt from the requirements prescribed in §§ 113.27 and 113.300(a), (b), and (c). Each serial shall meet the moisture requirements in § 113.300(e) and the special requirements prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Safety tests.* (1) Bulk or final container samples of completed product from each serial shall be tested for safety as prescribed in § 113.38.

(2) The prechallenge period of the potency test shall constitute a safety