§ 113.309 Bovine Parainfluenza Vaccine.

Bovine Parainfluenza\textsubscript{3} Vaccine shall be produced from virus-bearing cell culture fluids. 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. All serials of vaccine shall be prepared from the first through the tenth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in §113.300.

(b) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

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

(1) Twenty-five bovine parainfluenza, susceptible calves shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individual sera tested. Also, nasal specimens shall be collected for virus isolation attempts. The calves shall be considered susceptible if:

   (i) The results are negative at a 1:2 final serum dilution in a varying serum constant virus neutralization test with less than 500 TCID\textsubscript{50} of bovine parainfluenza\textsubscript{3} virus; and

   (ii) Shall be negative to bovine parainfluenza\textsubscript{3} virus isolation attempts from the nasal specimens on the day of injection.

(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. The 20 calves to be used as vaccinates shall be injected with a predetermined quantity of vaccine virus and the remaining five calves held as uninjected controls.

To confirm the dosage calculation, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) The vaccinates and controls shall be examined for clinical signs of respiratory disease and the body temperature taken and recorded on each of the first 14 consecutive days post-injection. The vaccinates shall be bled on day 6 ±2 days post-injection.

(4) Three to four weeks post-vaccination, all calves shall be bled for serum antibodies and nasal specimens shall be collected for PI\textsubscript{3} virus isolation. On the same day, all vaccinates and controls shall be given acceptable challenge PI\textsubscript{3} virus titrating at least 10\textsuperscript{7.0} TCID\textsubscript{50} per ml and the animals observed for 14 days. Two ml of the challenge virus shall be instilled in each nostril or shall be inhaled as an aerosol suspension. Upon request, challenge virus and instructions shall be furnished by Animal and Plant Health Inspection Service.

(5) Each animal shall be examined for clinical signs of respiratory disease and the body temperature recorded on each of the 14 consecutive days of the post-challenge observation period. Each day for at least the first 10 days post-challenge, nasal specimens for virus isolation attempts shall be taken. All animals shall be bled on day 6 ±2 days post-challenge, and all animals shall be bled at least once 14 to 28 days post-challenge for serum antibody studies.

(6) Satisfactory Test Criteria:

   (i) All virus isolations attempts shall be by culture and at least one subculture in PI\textsubscript{3} susceptible cells for a total of at least 14 days.

   (ii) Two to four weeks post-vaccination, at least 19 of the 20 vaccinates shall have PI\textsubscript{3} neutralizing antibody titers of at least 1:4 and all five controls shall be negative at 1:2 dilution. None of the post-vaccination serums collected from the vaccinates on day 6 ±2 days shall reveal serum neutralization antibody titers of 1:32 or greater based upon final dilution.
§ 113.310 Bovine Rhinotracheitis Vaccine.

Bovine Rhinotracheitis Vaccine 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 the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the tenth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in §113.300.

(b) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

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

(1) Twenty-five infectious bovine rhinotracheitis susceptible calves shall be used as test animals (20 vaccinates and five controls). Blood samples shall be drawn from these animals and individual serums tested. The calves shall be considered susceptible if the results are negative at a 1:2 final serum dilution by the virus plaque reduction method.

(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 per dose 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 the immunogenicity test but not less than $10^{6.2}$ TCID$_{50}$ per dose.