

by a prescribed test shall not be released.

(1) *Safety Test.* Samples of completed product from each serial or first subserial shall be tested for safety in calves as provided in §§ 113.41(a) and 113.41(b), except that the equivalent of two doses of vaccine shall be used and administered in the manner recommended on the label.

(2) *Bacterial count requirements.* Final container samples of completed product shall be tested for bacterial count using the method used in paragraph (b)(2) of this section. Two replicate titrations shall be conducted on each serial and subserial. Each sample shall be rehydrated with accompanying sterile diluent to the volume indicated on the label. To be eligible for release, each serial and subserial shall have a bacterial count sufficiently greater than that of the vaccine used in the immunogenicity test count per dose established to assure that, when tested at any time within the expiration period, each serial and subserial shall have a bacterial count at least two times greater than that used in the immunogenicity test.

[55 FR 35560, Aug. 31, 1990]

§ 113.70 *Pasteurella Multocida* Vaccine, Avian Isolate.

Pasteurella Multocida Vaccine, Avian Isolate, shall be prepared as a desiccated live culture of an avirulent or modified strain of *Pasteurella multocida*. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for vaccine production.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.64 and the requirements in this section.

(b) Each lot of Master Seed used for vaccine production shall be tested for immunogenicity in each species and for each serotype for which the Master Seed is claimed to give protection.

(1) Thirty *Pasteurella multocida* susceptible birds shall be used as test animals (20 vaccinates and 10 controls) for each bird species, route of administration, and serotype for which protection is claimed on the label.

(2) An arithmetic mean count of colony forming units from vaccine pro-

duced from the highest passage of Master Seed shall be established before the immunogenicity test is conducted. The 20 birds to be used as vaccinates shall be inoculated, as recommended on the label with a predetermined quantity of vaccine bacteria. The 10 control birds shall be held separately from the vaccinates. To confirm the dosage calculation, an arithmetic mean count shall be established by conducting five replicate titrations on a sample of the bacterial vaccine used. Only plates containing between 30 and 300 colonies shall be considered in a valid test.

(3) Not less than 14 days after vaccination, each of 20 vaccinates and each of 10 unvaccinated controls shall be challenged intramuscularly or by other methods acceptable to the Animal and Plant Health Inspection Service with a virulent *Pasteurella multocida* strain, for which protection is claimed, and observed daily for a 14 day post-challenge period.

(4) Eight or more of the unvaccinated controls must die for the test to be valid. If at least 16 of 20 of the vaccinates do not survive the 14-day postchallenge period, the Master Seed is unsatisfactory at the selected bacterial count.

(5) The Master Seed shall be retested for immunogenicity in 3 years and shall meet the criteria prescribed in paragraph (b)(4) of this section.

(c) *Test requirements for release.* Each serial and subserial shall meet the applicable requirements in §§ 113.8 and 113.64 and the requirements in this paragraph. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test.* Samples of completed product from each serial or first subserial shall be tested for safety.

(i) Ten birds of a species for which the vaccine is recommended shall be given the equivalent of 10 doses each of the vaccine and observed for 10 days. If the vaccine is recommended for more than one species, only one species needs to be tested.

(ii) If unfavorable reactions attributable to the vaccine occur during the observation period in two or more of the test birds, the serial is unsatisfactory.

(iii) If unfavorable reactions occur which are not attributable to the test vaccine, the test is inconclusive and may be repeated. If the results of the next test are not satisfactory, or if the test is not repeated, the serial shall be considered unsatisfactory.

(2) *Bacterial count requirements.* Final container samples of completed product shall be tested for bacterial count using the method used in paragraph (b)(2) of this section. Two replicate titrations shall be conducted on each serial and subserial. Each sample shall be rehydrated with accompanying sterile diluent to the volume indicated on the label. To be eligible for release, each serial and subserial shall have a bacterial count sufficiently greater than that of the vaccine used in the immunogenicity test count per dose established to assure that, when tested at any time within the expiration period, each serial and subserial shall have a bacterial count at least two times greater than that used in the immunogenicity test.

[55 FR 35560, Aug. 31, 1990, as amended at 59 FR 19633, Apr. 25, 1994; 64 FR 43044, Aug. 9, 1999]

§ 113.71 Chlamydia Psittaci Vaccine (Feline Pneumonitis), Live Chlamydia.

Chlamydia Psittaci Vaccine (Feline Pneumonitis), Live Chlamydia, shall be prepared from chlamydia-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed which has been established as pure, safe, and immunogenic shall be used 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 requirements prescribed in § 113.300 and the requirements in this section. Master Seed propagated in chicken embryos shall be tested for pathogens by the chicken embryo test prescribed in § 113.37. If found unsatisfactory by any prescribed test, the Master Seed shall not be used.

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

(1) Thirty feline pneumonitis susceptible cats shall be used as test animals (20 vaccinates and 10 controls). Blood samples shall be drawn and individual serum samples tested. The cats shall be considered suitable for use if all serums are negative for pneumonitis antibody in a complement fixation test or other test of equal sensitivity.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed shall be established before the immunogenicity test is conducted. The 20 cats used as vaccinates shall be administered a predetermined quantity of vaccine by the method to be recommended on the label and the remaining 10 cats shall be held as controls. To confirm the dosage calculations, five replicate titrations shall be conducted on a sample of the vaccine dilution used. If two doses are used, five replicate confirming titrations shall be conducted on each dose.

(3) Fourteen or more days after the final dose of vaccine, the vaccinates and controls shall each be challenged intranasally with a minimum of 10,000 yolk sac LD50 of virulent feline pneumonitis furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 28 days postchallenge. The rectal temperature of each animal shall be taken and the presence or absence of clinical signs noted and recorded each day.

(i) If less than 8 of 10 controls show clinical signs of feline pneumonitis infection other than fever, the test is inconclusive and may be repeated.

(ii) If a significant difference in clinical signs other than fever or chlamydia shedding cannot be demonstrated between vaccinates and controls using a scoring system approved by the Animal and Plant Health Inspection Service, the Master Seed is unsatisfactory.

(4) The Master Seed shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Either 10 vaccinates and 6 controls or 5 vaccinates and 3 controls shall be used in the retest.

(i) If less than five of six or three of three of the controls in the retest show clinical signs of feline pneumonitis infection other than fever, the test is inconclusive and may be repeated.