(i) **Chicken Embryo Test.** Each of 15 or more AE susceptible 5 or 6 day old embryos shall be injected in the yolk sac with 0.2 ml of the vaccine. For a valid test, at least 80 percent of the embryos shall survive for 48 hours post-inoculation (PI). Eleven to 13 days PI, all embryos surviving the 48 hour PI period shall be examined for gross lesions of AE; all these embryos shall be normal or the serial is unsatisfactory. Concurrently, five additional embryos from the same source shall be injected with live AE virus of the production strain to serve as positive controls. At least 4 of the 5 embryos shall show evidence of AE virus infection during the 11 to 13 day PI period or the test shall be considered inconclusive and repeated: Provided, That, if the test is not repeated, the serial shall be declared unsatisfactory.

(ii) **Chicken Test.** Each of 10 or more AE susceptible 7 day old chickens shall be injected intracerebrally with 0.1 ml vaccine each. The chickens shall be observed each day for 28 days. If any chickens show clinical signs of AE, the serial is unsatisfactory. Concurrently, 5 additional chickens from the same source shall be injected intracerebrally with live AE virus of the production strain to serve as positive controls. At least 4 of the 5 controls shall show evidence of AE virus infection during the observation period or the test shall be inconclusive and may be repeated: Provided, That, if the test is not repeated, the serial shall be unsatisfactory.

(b) **Potency test.** Bulk or final container samples of completed product from each serial or one subserial shall be tested. Ten or more AE-susceptible chickens (vaccinates), 4 weeks or older, properly identified and obtained from the same source and hatch, shall be injected as recommended on the label. At least 10 additional AE-susceptible chickens, properly identified and obtained from the same source and hatch shall be kept in isolation as controls.

(1) At least 28 days post-inoculation, the vaccinates and the controls shall be challenged intramuscularly with a virulent AE virus and the chickens observed each day for 21 days.

(2) If at least 80 percent of the controls do not show clinical signs of or die from AE infection, the test is inconclusive and may be repeated.

(3) If at least 80 percent of the vaccinates do not remain normal, the serial is unsatisfactory.
virus titrations. A mean relative potency value of the vaccine to be used in the host animal potency test must be established by at least five replicate potency tests conducted in accordance with the standard NIH test for potency in chapter 37 of "Laboratory Techniques in Rabies," Fourth Edition (1996), edited by F.X. Meslin, M.M. Kaplan, and H. Koprowski, World Health Organization, Geneva, Switzerland (ISBN 92 4 154479 1). The provisions of chapter 37 of "Laboratory Techniques in Rabies," Fourth Edition (1996), are the minimum standards for achieving compliance with this section and are incorporated by reference. These provisions state that the challenge virus standard to be used as the challenge in the NIH test and the reference vaccine for the test are available from the national control authority. In the United States, that authority is the Animal and Plant Health Inspection Service's Center for Veterinary Biologics Laboratory, located at 1920 Dayton Avenue, P.O. Box 844, Ames, IA 50010; phone (515) 337-6100; fax (515) 337-6120. This incorporation by reference was approved by the Director of the Federal Register in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies may be obtained from the World Health Organization Publications Center USA, 49 Sheridan Avenue, Albany, NY 12210. Copies may be inspected at the Animal and Plant Health Inspection Service's Center for Veterinary Biologics, Policy, Evaluation, and Licensing, 1920 Dayton Avenue, P.O. Box 844, Ames, IA 50010, or at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.

(2) The dose of vaccine to be used in the immunogenicity test shall be no more than the amount which, on the basis of The NIH Test For Potency, has been diluted to the proposed minimum acceptable potency value.  

(3) Test animals shall be uniform and have no neutralizing antibodies to rabies as determined by serum-neutralization (SN) tests.

(i) Twenty-five or more animals shall be used as vaccinates. Each shall be administered a dose of vaccine at the proposed minimum potency level and by the method specified in the Outline of Production.

(ii) Ten or more additional animals shall be held as controls.

(iii) On or about 30, 90, 180, 270, and 365 days postvaccination, all test animals shall be bled and individual serum samples tested for neutralizing antibodies to rabies virus.

(iv) All surviving test animals shall be challenged intramuscularly with virulent rabies virus furnished or approved by Animal and Plant Health Inspection Service 1 year after vaccinations, except as provided in (b)(4) of this section. The challenged animals shall be observed each day for 90 days as prescribed in §113.5(b). The brain of each test animal that dies following challenges shall be examined for rabies by the fluorescent antibody test or other method acceptable to Animal and Plant Health Inspection Service.

(v) Requirements for acceptance in challenge tests shall be death due to rabies in at least 80 percent of the controls while at least 22 of 25 or 26 of 30 or a statistically equivalent number of the vaccinates remain well for a period of 90 days.

(4) An alternative to challenging all surviving test animals in accordance with paragraph (b)(3)(iv) of this section may be used when the test animals are of species other than carnivores. Vaccinates shall be challenged at 1 year postvaccination. These shall include five vaccinates with the lowest SN titers at the 270th-day bleeding, five vaccinates with the lowest SN titers at the 365th-day bleeding, and all vaccinates with SN titers below 1:10 by the mouse SN test or below 1:16 by the rapid-fluorescent-focus-inhibition test at any bleeding. At least five SN-negative controls of each species shall be challenged at the same time as the vaccinates. All SN titers shall be titrated to an endpoint. All of the challenged vaccinates must remain well for a period of 90 days, and at least 80 percent of the controls must die of rabies for a satisfactory test without further
§ 113.210  Feline Calicivirus Vaccine, Killed Virus.  

Feline Calicivirus Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds 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.

(b) The Master Seed shall be tested for chlamydial agents as prescribed in §113.43.

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