

Unknown by six mice or more; or, if the total number of mice protected by the Standard exceeds the total number of mice protected by the Unknown by eight mice or more, the serial being tested is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than the minimum required in paragraph (c)(7) of this section, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP values obtained in the retests shall be determined. If the average RP is less than the required minimum, the serial is unsatisfactory. If the average RP obtained in the retests is equal to or greater than the required minimum, the following shall apply:

(i) If the RP obtained in the original test is one-third or less than the average RP obtained in the retests, the initial RP may be considered a result of test system error and the serial is satisfactory.

(ii) If the RP value obtained in the original test is more than one-third the average RP obtained in the retests, a new average shall be determined using the RP values obtained in all tests. If the new average is less than the minimum required in paragraph (c)(7) of this section, the serial is unsatisfactory.

[43 FR 25077, June 9, 1978, as amended at 48 FR 31009, July 6, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

#### KILLED VIRUS VACCINES

##### § 113.200 General requirements for killed virus vaccines.

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

(a) *Killing agent.* The vaccine virus shall be killed (inactivated) by an appropriate agent. The procedure involved may be referred to as inactivation. Suitable tests to assure complete

inactivation shall be written into the filed Outline of Production.

(b) *Cell culture requirements.* If cell cultures are used in the preparation of the vaccine, primary cells shall meet the requirements in §113.51 and cell lines shall meet the requirements in §113.52.

(c) *Purity tests*—(1) *Bacteria and fungi.* Final container samples of completed product from each serial shall be tested as prescribed in §113.26.

(2) *Avian origin vaccine.* 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.

(3) *Mycoplasma.* If the licensee cannot demonstrate that the agent used to kill the vaccine virus would also kill mycoplasma, each serial of the vaccine shall be tested for mycoplasma as prescribed in §113.28, prior to adding the killing agent. Material found to contain mycoplasma is unsatisfactory for use.

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

(d) *Safety tests.* Final container samples of completed product from each serial shall be tested for safety in guinea pigs as prescribed in §113.38 and for safety in mice as prescribed in §113.33: *Provided, That, vaccines recommended for use only in poultry are exempt from this requirement.*

(e) *Viricidal activity test.* Only serials tested for viricidal activity in accordance with the test provided in §113.35 and found satisfactory by such test shall be packaged as diluent for desiccated fractions in combination packages.

(f) *Formaldehyde content.* If formaldehyde is used as the killing agent, the residual free formaldehyde content must not exceed 0.74 grams per liter (g/

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L) as determined using the ferric chloride test.<sup>3</sup> Firms currently using tests for residual free formaldehyde content other than the ferric chloride test have until July 14, 2004 to update their Outline of Production to be in compliance with this requirement.

[39 FR 27428, July 29, 1974, as amended at 40 FR 23989, June 4, 1975; 43 FR 49528, Oct. 24, 1978. Redesignated at 55 FR 35562, Aug. 31, 1990; 68 FR 35283, June 13, 2003]

### § 113.201 Canine Distemper Vaccine, Killed Virus.

Canine Distemper 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 vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.200.

(b) The immunogenicity of vaccine prepared from the Master Seed Virus in accordance with the Outline of Production shall be established. Vaccine used for this test shall be at the highest passage from the Master Seed and prepared at the minimum preinactivation titer specified in the Outline of Production.

(1) Twenty-five canine distemper susceptible dogs (20 vaccinates and 5 controls) shall be used as test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine distemper to determine susceptibility. A constant virus-varying serum neutralization test in cell culture using 50 to 300 TCID<sub>50</sub> of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(i) The 20 dogs used as vaccinates shall be injected with one dose of vaccine by the method recommended on the label. If a second dose is recommended, the second dose shall be ad-

ministered at the time specified on the label.

(ii) At least 14 days after the last inoculation, the vaccinates and controls shall each be challenged intracerebrally with canine distemper virus furnished or approved by the Animal and Plant Health Inspection Service and observed each day for 21 days.

(iii) If at least four of the five controls do not die and the survivor, if any, does not show clinical signs of canine distemper, the test is inconclusive and may be repeated.

(iv) If at least 19 of the 20 vaccinated do not survive without showing clinical signs of canine distemper during the observation period, the Master Seed Virus is unsatisfactory.

(c) *Test requirements for release.* Each serial shall meet the applicable general requirements prescribed in § 113.200 and the special requirements for safety and potency provided in this section.

(1) *Safety test.* The vaccinates used in the potency test in paragraph (c)(2) of this section shall be observed each day during the postvaccination observation 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: *Provided*, That, if the test is not repeated, the serial is unsatisfactory.

(2) *Potency test—serum neutralization test.* Bulk or final container samples of completed product shall be tested for potency using five susceptible dogs (four vaccinates and one control) as the test animals. Blood samples drawn from each dog shall be individually tested for neutralizing antibody against canine distemper virus to determine susceptibility.

(i) A constant virus-varying serum neutralization test in tissue culture using 50 to 300 TCID<sub>50</sub> of virus shall be used. Dogs shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution.

(ii) *Vaccination.* Each of the four vaccinates shall be injected as recommended on the label. If two doses are recommended, the second dose

<sup>3</sup>The procedures for performing the ferric chloride test for residual free formaldehyde may be obtained from USDA, APHIS, Center for Veterinary Biologics-Laboratory, 1800 Dayton Road, P.O. Box 844, Ames, IA 50010.