§ 113.208 Avian Encephalomyelitis Vaccine, Killed Virus.

Avian Encephalomyelitis Vaccine (Killed Virus) shall be prepared from virus-bearing tissues or fluids obtained from embryonated chicken eggs. Each serial shall meet the general requirements prescribed in §113.200 and the requirements prescribed in this section. Any serial found unsatisfactory by any prescribed test shall not be released.

(a) Safety tests. (1) The prechallenge part of the potency test prescribed in paragraph (b) of this section shall constitute a safety test. If any of the vaccinates develop clinical signs of disease or die due to causes attributable to the product, the serial is unsatisfactory.

(2) An inactivation test for viable avian encephalomyelitis (AE) virus shall be conducted on each serial. The test shall be conducted using susceptible chicken embryos: Provided, That, if a non-embryo adapted virus is used for vaccine production, the test shall be conducted in susceptible chickens.

(1) 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

(b) Potency test. Bulk or final container samples of completed product from each serial shall be tested for potency in accordance with the two-stage test provided in this paragraph. For each fraction contained in the product—Eastern type, Western type, or Venezuelan type—the serological interpretations required in this test shall be made independently. A serial or subserial found unsatisfactory for any of the fractions shall not be released.

(1) For this test, a guinea pig dose shall be one-half the amount recommended on the label for a horse and shall be administered as recommended for a horse. Each of 10 healthy guinea pigs (vaccinates) shall be injected with two guinea pig doses with an interval of 14 to 21 days between doses. Two additional guinea pigs from the same source shall be held as controls.

(2) Fourteen to 21 days after the second injection, serum samples from each vaccinate and each control shall be tested by a plaque reduction, serum neutralization test using Vero 76 cells.

(3) If the control serum samples show a titer of 1:4 or greater for any fraction, the test is inconclusive for that fraction and may be repeated: Provided, That, if the test is not repeated, the serial is unsatisfactory.

(4) If two or three of the vaccinate serum samples show a titer of less than 1:40 for the Eastern type fraction, less than 1:40 for the Western type fraction, or less than 1:4 for the Venezuelan type fraction, the serial is unsatisfactory.

(5) If the second stage is used and four or more of the vaccinate serum samples show a titer of less than 1:40 for the Eastern type fraction or the Western type fraction, or less than 1:4 for the Venezuelan type fraction, the serial or subserial is unsatisfactory.

(6) The results shall be evaluated according to the following table:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Vaccinates</th>
<th>Failures for acceptance</th>
<th>Failures for rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1 or less</td>
<td>4 or more</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>3 or less</td>
<td>Do.</td>
</tr>
</tbody>
</table>

of the 5 embryos shall show evidence of
AE virus infection during the 11 to 13
day PI period or the test shall be con-
sidered inconclusive and repeated: Pro-
vided, That, if the test is not repeated,
the serial shall be declared unsatisfac-
tory.

(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 ob-
served 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 evi-
dence of AE virus infection during the
observation period or the test shall be
inconclusive and may be repeated: Pro-
vided, That, if the test is not repeated,
the serial shall be unsatisfactory.

(b) Potency test. Bulk or final con-
tainer 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 in-
jected as recommended on the label. At
least 10 additional AE-susceptible
chickens, properly identified and ob-
tained from the same source and hatch
shall be kept in isolation as controls.

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

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

(3) If at least 80 percent of the vac-
cinates do not remain normal, the se-
rial is unsatisfactory.

[39 FR 12958, Dec. 27, 1974, as amended at 40
FR 41088, Sept. 5, 1975. Redesignated at 55 FR
35562, Aug. 31, 1990, as amended at 56 FR
66786, Dec. 26, 1991]

§ 113.209 Rabies Vaccine, Killed Virus.

Rabies Vaccine (Killed Virus) shall
be prepared from virus-bearing cell cul-
tures or nerve tissues obtained from
animals that have developed rabies in-
fecion following injection with rabies
virus. Only Master Seed Virus which
has been established as pure, safe, and
immunogenic shall be used for pre-
paring the production seed virus for
vaccine production. All serials of vac-
cine shall be prepared from the first
through the fifth passage from the
Master Seed Virus.

(a) The Master Seed Virus shall meet
the applicable requirements prescribed
in §113.200 and the requirements pre-
scribed in this section.

(1) Each lot of Master Seed Virus
propagated in tissue cells of avian
origin shall also be tested for extra-
neous pathogens by procedures pre-
scribed in §113.37.

(2) Each lot of Master Seed Virus
propagated in primary cell cultures of
mouse or hamster origin or brain tis-
ues of mouse origin shall be tested for
lymphocytic choriomeningitis (LCM)

virus by the procedure prescribed in
§113.42. If LCM virus is detected, the
Master Seed Virus is unsatisfactory.

(b) The immunogenicity of vaccine
prepared with virus at the highest pas-
sage from the Master Seed shall be es-
tablished in each species for which the
vaccine is recommended. Tests shall be
conducted in accordance with a pro-
tocol filed with Animal and Plant
Health Inspection Service before initi-
ation of the tests. The vaccine shall be
prepared using methods prescribed in
the Outline of Production. If Rabies
Vaccine is to be in combination with
other fractions, the product to be test-
ed shall include all fractions to be test-
ed.

(1) The preinactivation virus titer
must be established as soon as possible
after harvest by at least five separate
virus titrations. A mean relative po-
tency 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 Tech-
niques in Rabies,” Fourth Edition
(1996), edited by F.X. Meslin, M.M.
Kaplan, and H. Koprowski, World
Health Organization, Geneva, Switzer-
land (ISBN 92 4 154479 1). The provisions
of chapter 37 of “Laboratory Tech-
niques in Rabies,” Fourth Edition
(1996), are the minimum standards for
achieving compliance with this section