NEWCASTLE DISEASE VACCINE (LIVE)

Vaccinum pseudopestis aviariae vivum

1. DEFINITION
Newcastle disease vaccine (live) is a preparation of a suitable strain of Newcastle disease virus (avian paramyxovirus 1). This monograph applies to vaccines intended for administration to chickens and/or other avian species for active immunisation.

2. PRODUCTION
2-1. PREPARATION OF THE VACCINE
The vaccine virus is grown in embryonated hens’ eggs or in cell cultures.

2-2. SUBSTRATE FOR VIRUS PROPAGATION
2-2-1. Embryonated hens’ eggs. If the vaccine virus is grown in embryonated hens’ eggs, they are obtained from flocks free from specified pathogens (SPF) (5.2.2).

2-2-2. Cell cultures. If the vaccine virus is grown in cell cultures, they comply with the requirements for cell cultures for production of veterinary vaccines (5.2.4).

2-3. SEED LOTS
2-3-1. Extraneous agents. The master seed lot complies with the tests for extraneous agents in seed lots (2.6.24). In these tests on the master seed lot, the organisms used are not more than 5 passages from the master seed lot at the start of the tests.

2-4. CHOICE OF VACCINE VIRUS
The vaccine virus shall be shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for the birds for which it is intended.

The following tests for intracerebral pathogenicity index (section 2-4-1), amino-acid sequence (section 2-4-2), safety (section 2-4-3), increase in virulence (section 2-4-4) and immunogenicity (section 2-4-5) may be used during the demonstration of safety and immunogenicity.

2-4-1. Intracerebral pathogenicity index. Use vaccine virus at the least attenuated passage level that will be present in a batch of the vaccine. Inoculate the vaccine virus into the allantoic cavity of embryonated hens' eggs, 9 to 11 days old, from an SPF flock (5.2.2). Incubate the inoculated eggs for a suitable period and harvest and pool the allantoic fluids. Use not fewer than ten 1-day-old chickens (i.e. more than 24 h but less than 40 h after hatching), from an SPF flock (5.2.2). Administer by the intracerebral route to each chick 0.05 ml of the pooled allantoic fluids containing not less than 10^7.0 EID₅₀, or, if this virus quantity cannot be achieved, not less than 10^6.0 EID₅₀. Observe the chickens at least daily for 8 days after administration and score them once every 24 h but less than 40 h after hatching, from an SPF flock (5.2.2). Administer by the intracerebral route to each chick 0.05 ml of the pooled allantoic fluids containing not less than 10^7.0 EID₅₀, or, if this virus quantity cannot be achieved, not less than 10^6.0 EID₅₀. Observe the chickens at least daily for 8 days after administration and score them once every 24 h. A score of 0 is attributed to a chicken if it is clinically normal, 1 if it shows clinical signs of disease and 2 if it is dead. The intracerebral pathogenicity index is the mean of the scores per chicken per observation over the 8 day period.

If an inoculum of not less than 10^6.0 EID₅₀ is used, the vaccine virus complies with the test if its intracerebral pathogenicity index is not greater than 0.5; if an inoculum of not less than 10^7.0 EID₅₀ but less than 10^6.0 EID₅₀ is used, the vaccine virus complies with the test if its intracerebral pathogenicity index is not greater than 0.4.

2-4-2. Amino-acid sequence. Determine the sequence of a fragment of RNA from the vaccine virus containing the region encoding for the F₀ cleavage site by a suitable method. The encoded amino-acid sequence is shown to be one of the following:

<table>
<thead>
<tr>
<th>Site</th>
<th>F1</th>
<th>F2</th>
<th>Cleavage site</th>
</tr>
</thead>
<tbody>
<tr>
<td>111</td>
<td>Gly</td>
<td>Gly</td>
<td>117</td>
</tr>
<tr>
<td>112</td>
<td>Gly</td>
<td>Gly</td>
<td>118</td>
</tr>
<tr>
<td>113</td>
<td>Lys</td>
<td>Lys</td>
<td>119</td>
</tr>
<tr>
<td>114</td>
<td>Gly</td>
<td>Gly</td>
<td></td>
</tr>
<tr>
<td>115</td>
<td>Arg</td>
<td>Arg</td>
<td></td>
</tr>
<tr>
<td>116</td>
<td>Arg</td>
<td>Arg</td>
<td></td>
</tr>
<tr>
<td>or</td>
<td>Gly</td>
<td>Gly</td>
<td>Leu Ile Gly</td>
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<tr>
<td>or</td>
<td>Gly</td>
<td>Glu</td>
<td>Gly Arg Gly Arg</td>
</tr>
<tr>
<td>or</td>
<td>Glu</td>
<td>Glu</td>
<td>Gly Arg Gly Arg</td>
</tr>
</tbody>
</table>

or equivalent with leucine at 117 and no basic amino acids at sites 111, 112, 114 and 115.

2-4-3. Safety. Carry out the test for each route and method of administration to be recommended for vaccination and in each avian species for which the vaccine is intended, using in each case birds not older than the youngest age to be recommended for vaccination. Use vaccine virus at the least attenuated passage level that will be present between the master seed lot and a batch of the vaccine. For tests in chickens, use not fewer than 20 chickens, from an SPF flock (5.2.2). For species other than the chicken, use not fewer than 20 birds that do not have antibodies against Newcastle disease virus. Administer to each bird a quantity of the vaccine virus equivalent to not less than 10 times the maximum virus titre likely to be contained in 1 dose of the vaccine. Observe the birds at least daily for 21 days. The test is not valid if more than 30 per cent of the birds show abnormal clinical signs or die from causes not attributable to the vaccine virus. The vaccine virus complies with the test if no birds shows notable clinical signs of Newcastle disease or dies from causes attributable to the vaccine virus.

2-4-4. Increase in virulence. The test for increase in virulence consists of the administration of the vaccine virus at the least attenuated passage level that will be present between the master seed lot and a batch of the vaccine to a group of 5 birds not more than 2 weeks old, sequential passages, 5 times where possible, to further similar groups and testing of the final recovered virus for increase in virulence. If the properties of the vaccine virus allow sequential passage to 5 groups via natural spreading, this method may be used, otherwise passage as described below is carried out and the maximally passaged virus that has been recovered is tested for increase in virulence. Care must be taken to avoid contamination by virus from previous passages. Carry out the test in a target species, using the chicken if it is one of the target species. For the test in chickens, use chickens from an SPF flock (5.2.2). For other species, carry out the test in birds that do not have antibodies against Newcastle disease virus. Administer by eye-drop a quantity of the vaccine virus that will allow recovery of virus for the passages described below. Observe the birds for the period shown to correspond to maximum replication of the vaccine virus, kill them and prepare a suspension from the brain of each bird and from a suitable organ depending on the tropism of the strain (for example, mucosa of the entire trachea, intestine, pancreas); pool the samples. Administer 0.05 ml of the pooled samples by eye-drop to each of 5 other birds of the same species, age and origin. Carry out this passage operation not fewer than 5 times; verify the presence of the virus at each passage. If the virus is not found at a passage level, carry out a second series of passages.

A. Carry out the test for intracerebral pathogenicity index (section 2-4-1) using unpassaged vaccine virus and the maximally passaged virus that has been recovered.
B. Carry out the test for amino-acid sequence (section 2-4-2) using unpassaged vaccine virus and the maximally passaged virus that has been recovered.

C. Carry out the test for safety (section 2-4-3) using unpassaged vaccine virus and the maximally passaged virus that has been recovered. Administer the virus by the route to be recommended for vaccination likely to be the least safe and to the avian species for which the vaccine is intended that is likely to be the most susceptible to Newcastle disease.

The vaccine virus complies with the test if, in the tests 2-4-A, 2-4-B and 2-4-C, no indication of increase in virulence of the maximally passaged virus compared with the unpassaged virus is observed. If virus is not recovered at any passage level in the first and second series of passages, the vaccine virus also complies with the test.

2-4-5. Immunogenicity. For each avian species for which the vaccine is intended, a test is carried out for each route and method of administration to be recommended using in each case birds not older than the youngest age to be recommended for vaccination. The quantity of the vaccine virus administered to each bird is not greater than the minimum titre to be stated on the label and the virus is at the most attenuated passage level that will be present in a batch of the vaccine.

2-4-5-1. Vaccines for use in chickens. Use not fewer than 30 chickens of the same origin and from an SPF flock (5.2.2). Vaccinate by a recommended route not fewer than 20 chickens. Maintain not fewer than 10 chickens as controls. Challenge each chicken after 21 days by the intramuscular route with not less than 10^6.5 embryo LD50 of the Herts (Weybridge 33/56) strain of Newcastle disease virus. Observe the chickens at least daily for 14 days after challenge. Record the deaths and the number of surviving chickens that show clinical signs of disease. The test is not valid if 6 days after challenge fewer than 100 per cent of the control chickens have died or if during the period between vaccination and challenge more than 10 per cent of the vaccinated or control chickens show abnormal clinical signs or die from causes not attributable to the vaccine. The vaccine virus complies with the test if during the observation period after challenge not fewer than 90 per cent of the vaccinated chickens survive and show no notable clinical signs of Newcastle disease.

2-4-5-2. Vaccines for use in avian species other than the chicken. Use not fewer than 30 birds of the species for which the vaccine is intended for Newcastle disease, of the same origin and that do not have antibodies against avian paramyxovirus 1. Vaccinate by a recommended route not fewer than 20 birds. Maintain not fewer than 10 birds as controls. Challenge each bird after 21 days by the intramuscular route with a sufficient quantity of virulent avian paramyxovirus 1. Observe the birds at least daily for 21 days after challenge. Record the deaths and the surviving birds that show clinical signs of disease. The test is not valid if:
- during the observation period after challenge fewer than 90 per cent of the control birds die or show severe clinical signs of Newcastle disease,
- or if during the period between the vaccination and challenge more than 10 per cent of the vaccinated or control birds show abnormal clinical signs or die from causes not attributable to the vaccine.

The vaccine virus complies with the test if during the observation period after challenge not fewer than 90 per cent of the vaccinated birds survive and show no notable clinical signs of Newcastle disease. For species where there is published evidence that it is not possible to achieve this level of protection, the vaccine complies with the test if there is a significant reduction in morbidity and mortality of the vaccinated birds compared with the control birds.

3. BATCH TESTS

3-1. Identification

3-1-1. Identification of the vaccine virus. The vaccine, diluted if necessary and mixed with a monospecific Newcastle disease virus antisera, no longer provokes haemagglutination of chicken red blood cells or infects embryonated hens’ eggs from an SPF flock (5.2.2) or susceptible cell cultures (5.2.4) into which it is inoculated.

3-1-2. Identification of the virus strain. The strain of vaccine virus is identified by a suitable method, for example using monoclonal antibodies.

3-2. Bacteria and fungi

Vaccines intended for administration by injection comply with the test for sterility prescribed in the monograph Vaccines for veterinary use (0062).

Vaccines not intended for administration by injection either comply with the test for sterility prescribed in the monograph Vaccines for veterinary use (0062) or with the following test: carry out a quantitative test for bacterial and fungal contamination; carry out identification tests for microorganisms detected in the vaccine; the vaccine does not contain pathogenic microorganisms and contains not more than 1 non-pathogenic microorganism per dose.

Any liquid supplied with the vaccine complies with test for sterility prescribed in the monograph Vaccines for veterinary use (0062).

3-3. Mycoplasmas. The vaccine complies with the test for mycoplasmas (2.6.7).

3-4. Extraneous agents. The vaccine complies with the tests for extraneous agents in batches of finished product (2.6.25).

3-5. Safety. For vaccines recommended for use in chickens, use not fewer than 10 chickens from an SPF flock (5.2.2) and of the youngest age recommended for vaccination. For vaccines recommended for use only in avian species other than the chicken, use not fewer than 10 birds of the species likely to be most sensitive to Newcastle disease, that do not have antibodies against Newcastle disease virus and of the minimum age recommended for vaccination. Administer to each bird by eye-drop, or parenterally if only parenteral administration is recommended, 10 doses of the vaccine in a volume suitable for the test. Observe the birds at least daily for 21 days. The test is not valid if more than 20 per cent of the birds show abnormal clinical signs or die from causes not attributable to the vaccine. The vaccine complies with the test if no bird shows notable clinical signs of disease or dies from causes attributable to the vaccine.

3-6. Virus titre. Titrate the vaccine virus by inoculation into embryonated hens’ eggs from an SPF flock (5.2.2) or into suitable cell cultures (5.2.4). The vaccine complies with the test if 1 dose contains not less than the minimum virus titre stated on the label.

3-7. Potency. Depending on the indications, the vaccine complies with 1 or both of the tests prescribed under Immunogenicity (section 2-4-5) when administered according to the recommended schedule by a recommended route and method. If the test in section 2-4-5-2. Vaccine for use in avian species other than the chicken is conducted and the vaccine is recommended for use in more than 1 avian species, the test is carried out with birds of that species for which the vaccine is recommended which is likely to be the most susceptible to avian paramyxovirus 1. It is not necessary
to carry out the potency test for each batch of the vaccine if it has been carried out on a representative batch using a vaccinating dose containing not more than the minimum virus titre stated on the label.

The vaccine complies with the test if no animal shows abnormal local reactions or clinical signs of disease or dies from causes attributable to the vaccine. In addition, if the vaccine is intended for use in pregnant ewes, no significant effects on the pregnancy and offspring are demonstrated.

**B. The animals used for the field trials are also used to evaluate safety.** Carry out a test in each category of animals for which the vaccine is intended. Use not fewer than 3 groups of 20 animals with corresponding groups of not fewer than 10 controls in 3 different locations. Examine the injection sites for local reactions after vaccination. Record body temperatures the day before vaccination, at vaccination and on the 2 days following vaccination. The vaccine complies with the test if no animal shows abnormal local or systemic reactions or clinical signs of disease or dies from causes attributable to the vaccine. The average body temperature increase for all animals does not exceed 1.5 °C and no animal shows a rise greater than 2 °C. In addition, if the vaccine is intended for use in pregnant ewes, no significant effects on the pregnancy and offspring are demonstrated.

**Immunogenicity.** As part of the studies to demonstrate the suitability of the vaccine with respect to immunogenicity, the test described under Potency may be carried out for each proposed route of administration and using vaccine of minimum potency.

### BATCH TESTING

**Batch potency test.** The test described under Potency is not carried out for routine testing of batches of vaccine. It is carried out, for a given vaccine, on one or more occasions, as decided by or with the agreement of the competent authority. Where the test is not carried out, a suitable validated batch potency test is carried out, the criteria for acceptance being set with reference to the results obtained with a batch of vaccine that has given satisfactory results in the test described under Potency.

**Bacterial endotoxins.** A test for bacterial endotoxins (2.6.14) is carried out on the final lot or, where the nature of the adjuvant prevents performance of a satisfactory test, on the bulk antigen or the mixture of bulk antigens immediately before addition of the adjuvant. The maximum acceptable amount of bacterial endotoxins is that found for a batch of vaccine that has been shown satisfactory in safety test A given under Choice of vaccine composition or in the safety test described under Tests, carried out using 10 animals. Where the latter test is used, note the maximum temperature increase for each animal; the average body temperature increase for all animals does not exceed 1.5 °C. The method chosen for determining the amount of bacterial endotoxin present in the vaccine batch used in the safety test for determining the maximum acceptable level of endotoxin is used subsequently for testing of each batch.

### IDENTIFICATION

When injected into healthy seronegative animals, the vaccine stimulates the production of specific antibodies against the serovars of *P. trehalosi* and/or against the leucotoxin present in the vaccine.

### TESTS

**Safety.** Use 2 sheep of the minimum age recommended for vaccination or, if not available, of an age as close as possible to the minimum recommended age, and that have not been vaccinated against Pasteurella. Administer a double dose of vaccine to each animal by a recommended route. Observe the animals for 14 days. Record body temperature the day before vaccination, at vaccination, 2 h, 4 h and 6 h later and...