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Newcastle Disease Vaccine, Inactivated



[General Notices](#)

(*Newcastle Disease Vaccine (Inactivated)*, Ph. Eur. monograph 0870)

Ph Eur

1 DEFINITION

Newcastle disease vaccine (inactivated) (also known as avian paramyxovirus 1 vaccine (inactivated) for vaccines intended for some species) is a preparation of a suitable strain of Newcastle disease virus (avian paramyxovirus 1), inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of birds against Newcastle disease.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The vaccine virus is grown in embryonated hens' eggs or in cell cultures. The virus harvest is inactivated. The vaccine may be adjuvanted.

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 healthy flocks ([5.2.13](#)).

2-2-2 Cell cultures

If the vaccine virus is grown in cell cultures, they comply with the requirements for cell cultures for the production of vaccines for veterinary use ([5.2.4](#)).

2-3 CHOICE OF VACCINE COMPOSITION

The vaccine is shown to be satisfactory with respect to safety ([5.2.6](#)) and efficacy ([5.2.7](#)) for each species and category of birds for which it is intended.

The following tests for safety (section 2-3-1) and immunogenicity (section 2-3-2) may be used during the demonstration of safety and efficacy.

2-3-1 Safety

The test is carried out for each route of administration to be recommended for vaccination and for each avian species for which the vaccine is intended. Use a batch of vaccine containing not less than the maximum potency that may be expected in a batch of vaccine.

For each test performed in birds younger than 3 weeks of age, use not fewer than 10 birds not older than the minimum age to be recommended for vaccination. For each test performed in birds older than 3 weeks of age, use not fewer than 8 birds not older than the minimum age to be recommended for vaccination. In the case of chickens, use chickens from a flock free from specified pathogens (SPF) (5.2.2) and if the vaccine is used for species other than chickens, they have not been vaccinated and do not have antibodies against Newcastle disease virus. Administer by a route and method to be recommended to each bird 1 dose of vaccine. Observe the birds at least daily for at least 14 days after the last administration of the vaccine.

The test is not valid if more than 10 per cent of the birds younger than 3 weeks of age show abnormal signs of disease or die from causes not attributable to the vaccine. For birds older than 3 weeks of age, the test is not valid if non-specific mortality occurs.

The vaccine complies with the test if no bird shows abnormal signs of disease or dies from causes attributable to the vaccine.

2-3-2 Immunogenicity

A test is carried out for each route and method of administration to be recommended; the vaccine administered to each bird is of minimum potency.

For chickens, the test for vaccines for use in chickens (section 2-3-2-1) is suitable for demonstrating immunogenicity. For other species of birds (for example, pigeons or turkeys), the test for vaccines for use in species other than chickens (section 2-3-2-2) is suitable for demonstrating immunogenicity.

2-3-2-1 Vaccines for use in chickens. Use not fewer than 70 chickens, 21-28 days old, of the same origin and from an SPF flock (5.2.2). For vaccination, use not fewer than 3 groups, each of not fewer than 20 chickens. Choose a number of different volumes of the vaccine corresponding to the number of groups: for example, volumes equivalent to 1/25, 1/50 and 1/100 of a dose. Allocate a different volume to each vaccination group. Vaccinate each chicken by the intramuscular route with the volume of vaccine allocated to its group. Maintain not fewer than 10 chickens as controls. Challenge each chicken after 17-21 days by the intramuscular route with 6 log₁₀ embryo LD₅₀ of the Herts (Weybridge 33/56) strain of avian paramyxovirus 1. Observe the chickens at least daily for 21 days after challenge. At the end of the observation period, calculate the PD₅₀ by standard statistical methods from the number of chickens that survive in each vaccinated group without showing any signs of Newcastle disease during the 21 days.

The test is invalid unless all the control birds die within 6 days of challenge.

The vaccine complies with the test if the smallest dose stated on the label corresponds to not less than 50 PD₅₀ and the lower confidence limit is not less than 35 PD₅₀ per dose. If the lower confidence limit is less than 35 PD₅₀ per dose, repeat the test; the vaccine must be shown to contain not less than 50 PD₅₀ in the repeat test.

2-3-2-2 Vaccines for use in species other than chickens. Use not fewer than 30 birds of the target species, of the same origin and of the same age, that do not have antibodies against avian paramyxovirus 1. Vaccinate in accordance with the recommendations for use not fewer than 20 birds. Maintain not fewer than 10 birds as controls. Challenge each bird after 4 weeks by the intramuscular route with a sufficient quantity of virulent avian paramyxovirus 1.

The test is not valid if serum samples obtained at the time of the first vaccination show the presence of antibodies against avian paramyxovirus 1 in either vaccinates or controls, or if tests carried out at the time of challenge show such antibodies in controls.

The test is not valid if fewer than 70 per cent of the control birds die or show serious signs of Newcastle disease.

The vaccine complies with the test if not fewer than 90 per cent of the vaccinated birds survive and show no serious signs of avian paramyxovirus 1 infection.

2-4 MANUFACTURER'S TESTS

2-4-1 Residual live virus

The test is carried out in suitable cell cultures or in embryonated eggs, whichever is the most sensitive for the vaccine strain. The quantity of inactivated virus harvest used in the test is equivalent to not less than 10 doses of vaccine.

A. If cell cultures are more sensitive for the vaccine strain, carry out a suitable test for residual live virus using 2 passages of cell culture. The inactivated virus harvest complies with the test if no live virus is detected.

B. If embryonated eggs are more sensitive for the vaccine strain, inject 0.2 mL of inactivated virus harvest into the allantoic cavity of each of 10 embryonated hen eggs that are 9-11 days old and from SPF flocks ([5.2.2](#)) (SPF eggs), and incubate. Observe for 6 days and pool separately the allantoic fluid from eggs containing live embryos and that from eggs containing dead embryos, excluding those dying within 24 h of the injection. Examine embryos that die within 24 h of injection for the presence of Newcastle disease virus: the vaccine does not comply with the test if Newcastle disease virus is found.

Inject into the allantoic cavity of each of 10 SPF eggs, 9-11 days old, 0.2 mL of the pooled allantoic fluid from the live embryos and, into each of 10 similar eggs, 0.2 mL of the pooled fluid from the dead embryos and incubate for 5-6 days. Test the allantoic fluid from each egg for the presence of haemagglutinins using chicken erythrocytes. Examine embryos that die within 24 h of injection for the presence of Newcastle disease virus: the vaccine does not comply with the test if Newcastle disease virus is found.

The inactivated virus harvest complies with the test if there is no evidence of haemagglutinating activity and if not more than 20 per cent of the embryos die at either stage. If more than 20 per cent of the embryos die at one of the stages, repeat that stage; the inactivated virus harvest complies with the test if there is no evidence of haemagglutinating activity and not more than 20 per cent of the embryos die at that stage.

Antibiotics may be used in the test to control extraneous bacterial infection.

2-4-2 Batch potency test

It is not necessary to carry out the potency test (section 3-3) for each batch of vaccine if it has been carried out using a batch of vaccine with a minimum potency. Where the test is not carried out, an alternative validated method is used, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency. The following tests may be used. Wherever possible, carry out the test for antigen content (section 2-4-2-1) together with the test for adjuvant (section 2-4-2-2).

Vaccines for use in chickens The test for antigen content (section 2-4-2-1) together with the test for adjuvant (section 2-4-2-2) may be carried out; if the nature of the product does not allow valid results to be obtained with these tests, or if the vaccine does not comply, the test for serological assay (section 2-4-2-3) may be carried out. If the vaccine does not comply with the latter test, the test for vaccines for use in chickens (section 2-3-2-1) may be carried out. A test using fewer than 20 birds per group and a shorter observation period after challenge may be used if this has been shown to give a valid potency test.

Vaccines for use in species other than chickens Carry out a suitable test for which a satisfactory correlation has been established with the test for vaccines for use in species other than chickens (section 2-3-2-2), the criteria for acceptance being set with reference to a batch that has given satisfactory results in the latter test. A test in chickens from an SPF flock ([5.2.2](#)) consisting of a measure of the serological response to graded amounts of vaccine (for example, 1/25, 1/50 and 1/100 of a dose with serum sampling 17-21 days later) may be used. Alternatively, the test for antigen content (section 2-4-2-1) together with the test for adjuvant (section 2-4-2-2) may be conducted if shown to provide a valid potency test.

2-4-2-1 Antigen content. The relative antigen content is determined by comparing the content of haemagglutinin-neuraminidase antigen per dose of vaccine with a haemagglutinin-neuraminidase antigen reference preparation, by enzyme-linked immunosorbent assay ([2.7.1](#)). For this comparison, [Newcastle disease virus reference antigen BRP](#), [Newcastle disease virus control antigen BRP](#), [Newcastle disease virus coating antibody BRP](#) and [Newcastle disease virus conjugated detection antibody BRP](#) are suitable. Before estimation, the antigen may be extracted from the emulsion using [isopropyl myristate R](#) or another suitable method. The vaccine complies with the test if the estimated antigen content is not significantly lower than that of a batch that has been found to be satisfactory with respect to immunogenicity (section 2-3-2).

2-4-2-2 Adjuvant. If the immunochemical assay (section 2-4-2-1) is performed and if the vaccine is adjuvanted, the adjuvant is tested by suitable physical and chemical methods. For oil-adjuvanted vaccines, the adjuvant is tested in accordance with the general monograph [Vaccines for veterinary use \(0062\)](#). If the adjuvant cannot be adequately characterised, the antigen content determination cannot be used as the batch potency test.

2-4-2-3 Serological assay. Use not fewer than 13 chickens, 21-28 days old, of the same origin and from an SPF flock ([5.2.2](#)). Vaccinate by the intramuscular route not fewer than 10 chickens with a volume of the vaccine equivalent to 1/50 of a dose. Maintain not fewer than 3 chickens as controls. Collect serum samples from each chicken after 17-21 days. Measure the antibody levels in the sera by the haemagglutination-inhibition (HI) test using the technique described below or an equivalent technique with the same numbers of haemagglutinating units and red blood cells. The test system used must include negative and positive control sera, the latter having an HI titre of $5.0 \log_2$ to $6.0 \log_2$. The vaccine complies with the test if the mean HI titre of the vaccinated group is equal to or greater than $4.0 \log_2$ and that of the unvaccinated group is $2.0 \log_2$ or less. If the HI titres are not satisfactory, carry out the test for vaccines for use in chickens (section 2-3-2-1).

Haemagglutination inhibition

Inactivate the test sera by heating at 56 °C for 30 min. Add 25 µL of inactivated serum to the first row of wells in a microplate. Add 25 µL of a buffered 9 g/L solution of [sodium chloride R](#) at pH 7.2-7.4 to the rest of the wells. Prepare twofold dilutions of the sera across the plate. To each well add 25 µL of a suspension containing 4 haemagglutinating units of inactivated Newcastle disease virus. Incubate the plate at 4 °C for 1 h. Add 25 µL of a 1 per cent V/V suspension of red blood cells collected from chickens that are 3-4 weeks old and free from antibodies against Newcastle disease virus. Incubate the plate at 4 °C for 1 h. The HI titre is equal to the highest dilution that produces complete inhibition.

3 BATCH TESTS

3-1 Identification

The vaccine contains the antigen or antigens stated under Definition.

3-2 Bacteria and fungi

The vaccine, including where applicable the diluent supplied for reconstitution, complies with the test for sterility prescribed in the general monograph [Vaccines for veterinary use \(0062\)](#).

3-3 Potency

The vaccine complies with the requirements of the test prescribed under Immunogenicity (section 2-3-2) when administered by a recommended route and method.