DRAFT REVISED MONOGRAPH FOR COMMENTS

This draft revised monograph contain text for inclusion in the Indian Pharmacopoeia (IP). The content of this draft document is not final, and the text may be subject to further revisions prior to publication in the IP. This draft does not necessarily represent the decisions or the stated policy of the IP or Indian Pharmacopoeia Commission (IPC).

Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Comments received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in/ biologics-ipc@gov.in **before the last date for comments.**

Document History and Schedule for the Adoption Process

Description	**Details**
Document version	1.0
First Draft published on IPC website for public comments	1st November 2024
Last Date for Comments	15th December 2024
Monograph Revision proposed for Inclusion in	IP 2026
Tentative effective date of proposed amendment	January, 2026
Draft revision published on IPC website for public comments	NA
Further follow-up action as required.	

Ranikhet Disease Vaccine, Inactivated

Newcastle Disease Vaccine, Inactivated

Ranikhet Disease Vaccine, Inactivated consists of an emulsion or a suspension of a suitable strain of Newcastle disease virus (avian paramyxovirus 1) that has been inactivated in such a manner that immunogenic activity is retained.

This monograph applies to vaccines intended for the active immunization of birds against Newcastle disease.

Production

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.

Substrate for virus propagation

Embryonated hens' eggs

If the vaccine virus is grown in embryonated hens' eggs, they are obtained from healthy flocks (2.7.7.).

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 (2.7.13)

Seed lots

The master seed lot complies with the tests for extraneous agents (2.7.10).

Identification. Vaccine prepared from Master Seed, injected into susceptible healthy chick stimulates the production of specific antibodies against Newcastle disease virus.

Choice of vaccine composition

The vaccine is shown to be satisfactory with respect to safety (2.7.17) and efficacy (2.7.12) for each species and category of birds for which it is intended. The following tests for safety and immunogenicity may be used during the demonstration of safety and efficacy.

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. Use not fewer than 10 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) (2.7.7) or healthy susceptible chickens (2.7.18) 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 double dose of vaccine. Observe the birds at least daily for at least 21 days after the last administration of the vaccine. 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.

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 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 is suitable for demonstrating immunogenicity.

Vaccines for use in chickens

Use not fewer than 70 chickens, 21-28 days old, of the same origin and from an SPF flock (2.7.7) or healthy susceptible chickens (2.7.18). 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 5 $\log_{10}$ embryo LD_{50} of the virulent 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 PD_{50} 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 PD_{50} and the lower confidence limit is not less than 35 PD_{50} per dose. If the lower confidence limit is less than 35 PD_{50} per dose, repeat the test; the vaccine must be shown to contain not less than 50 PD_{50} in the repeat test.

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.

Manufacturer's test

Identification. When injected into susceptible healthy chick; the vaccine stimulates the production of specific antibodies against Newcastle disease virus. Alternatively, identification on the final bulk lot by molecular techniques is acceptable and can be used in the routine bulk lot release. Once this test is performed on the final bulk, it may be omitted on the final product.

Residual live virus

The test is carried out in embryonated eggs or suitable cell cultures (2.7.13), 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. The inactivated virus harvest complies with the test if no live virus is detected

A test for residual live virus is carried out to confirm inactivation of Newcastle disease virus.

Inject 10 doses into the allantoic cavity of each of 10 embryonated hen eggs that are 9-11 days old and from SPF flocks (2.7.7) 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 hours 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. The vaccine 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 vaccine 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.

Potency test. It is not necessary to carry out the potency test for each batch of vaccine if it has been carried out using a batch of vaccine with a minimum potency prepared from the same master seed lot. 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 together with the test for adjuvant

Vaccines for use in chickens

The test for antigen content together with the test for adjuvant 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 may be carried out. If the vaccine does not comply with the latter test, the test for vaccines for use in chickens may be carried out. A test using fewer than 20 birds per group and 7 days 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, 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 (2.7.7) 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 together with the test for may be conducted if shown to provide a valid potency test.

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. 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

Adjuvant

If the immunochemical assay (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 Veterinary Vaccines General

requirement. If the adjuvant cannot be adequately characterised, the antigen content determination cannot be used as the batch potency test.

Serological assay

Use not fewer than 15 chickens, 21-28 days old, of the same origin and from an SPF flock (2.7.7) or healthy susceptible chickens **(**2.7.18**)**. 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 5 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.

Haemagglutination inhibition

Inactivate the test sera by heating at 56 ° for 30 minutes. Add 25 µl of inactivated serum to the first row of wells in a microtitre plate. 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° for 1 hour. 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° for 1 hour. The HI titre is equal to the highest dilution that produces complete inhibition.

Batch tests

Identification. Vaccine complies with the requirements of the tests mentioned under production. Alternatively, identification on the final bulk by molecular techniques is acceptable and can be used for the routine batch release tests.

Bacterial and fungal contamination (2.2.11). The vaccine complies with the test for sterility

Safety. Inject ten SPF chickens (2.7.7, Table 3) or healthy susceptible chickens of the age stated on the label with twice the dose and by the route stated on the label. Observe the birds for 21 days. No abnormal local or systemic reactions are observed.
Note: General Requirements shall be referred regarding omission of the batch safety test.

Potency. The vaccine complies with the requirements of the test prescribed under manufacturer's test when administered by a recommended route and method.

Labelling

The label must state that (1) the vaccine is for veterinary use only; (2) the recommended routes of administration; (3) the instructions for use such as "the preparation should be shaken well before use. 4) The animal species for which the vaccine is intended. (5) storage temperatures; (6) Batch Number, Manufacturing date and expiry date; (7) Total volume or number of doses; (8) Strain of virus used in preparing the vaccine.