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

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Marek's Disease Vaccine, Live

Marek's Disease, Frozen / Freeze dried / Cell Associated Vaccine, Live is a preparation of a suitable serotype(s) of Marek's Disease Virus (Avian Herpes Virus) or combinations their of. This monograph applies to vaccines intended for administration to chickens and/or chicken embryos for active immunization against Marek's Disease.

Production

Preparation of the vaccine

The vaccine virus is grown in cell cultures obtained from SPF (2.7.7) eggs. If the vaccine contains more than one type of virus, the different types are grown separately. The vaccine may be freeze-dried or stored in liquid nitrogen.

Substrate for virus propagation

Cell cultures. Cell culture derived from SPF eggs (2.7.7) from SPF hens (2.7.7) eggs or any suitable cell line.

Seed lots

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

Identification

Carry out either the test A or B.

A. The vaccine on inoculation in susceptible cell culture derived from SPF embryos causes cytopathic effects typical of Marek's disease virus.

B. When mixed with a specific avian herpes virus antiserum, the vaccine loses its capability to produce cytopathic effects or plaques in susceptible cell cultures derived from SPF embryos.

Choice of vaccine virus

The vaccine virus shall be shown to be satisfactory with respect to safety (2.7.17) and efficacy (2.7.12) for the chickens and/or chicken embryos for which it is intended. The tests shown below for residual pathogenicity of the strain, test for reversal to virulence and immunogenicity may be used during the demonstration of safety and efficacy.

Safety

Residual pathogenicity of the strain

Carry out the test for the route to be recommended for vaccination that is likely to be the least safe and in the category of chickens for which the vaccine is intended that is likely to be the most susceptible for Marek's disease. Carry out the test in chickens if the vaccine is intended for chickens; carry out the test in chicken embryos if the vaccine is intended for chicken embryos; carry out the test in chickens and in chicken embryos if the vaccine is intended for both.

Use vaccine virus at the least attenuated passage level that will be present between the master seed lot and a batch of the vaccine.

Vaccines intended for use in chickens

Use not fewer than 80 one day old chickens from a flock free from specified pathogens (SPF) (2.7.7). Divide them randomly into 2 groups of not fewer than 40 chickens and maintain the groups separately. Administer by a suitable route to each chicken of one group-I 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. Administer by a suitable route to each chicken of the other group -II a quantity of virulent Marek's disease virus that will cause mortality and/or severe macroscopic lesions of Marek's disease in not fewer than 70 per cent of the effective number of chickens within 70 days (initial number reduced by the number that die within the first 7 days of the test).

Vaccines intended for use in chicken embryos

Use not fewer than 150 embryonated eggs from an SPF flock) (2.7.7). Divide them randomly into 3 groups of not fewer than 50 embryonated eggs and maintain the groups separately but under identical incubation conditions. Not later than the recommended day of vaccination, administer by the method to be recommended to each embryonated egg of one group-I 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. Administer by a suitable route to each embryonated egg of another group -II a quantity of virulent Marek's disease virus that will cause mortality and/or severe macroscopic lesions of Marek's disease in not fewer than 70 per cent of the effective number of hatched chickens within 70 days (initial number reduced by the number that die within the first 7 days after hatching). Keep the last group -III non-inoculated. The test is not valid if there is a significant difference in hatchability between groups I and III and the hatchability in any of the 3 groups is less than 80 per cent.

Provided that the chickens and chicken embryos are derived from the same flock, a common control group for in *ovo* and parenteral administration can be used. Irrespective of whether the vaccine was administered to chickens or chicken embryos, observe the chickens of group II at least daily for 70 days and those of group I at least daily for 120 days.

The test is not valid if one or more of the following apply:

– more than 10 per cent of the chickens in any of the 3 groups die within the first 7 days;

– fewer than 70 per cent of the effective number of chickens in group II show macroscopic lesions of Marek's disease;

The vaccine virus complies with the test if:

the number of surviving chickens in Group 1 is not fewer than 80% of effective number after 120 days of observation period

No notable clinical signs or macroscopic lesions of Marek's disease are observed during this observation period or no chicken dies from causes attributable to the vaccine virus.

Test for Reversion to virulence

The test for reversion to virulence is required for Marek's disease virus vaccine strains but not for turkey herpes virus vaccine strains, which are naturally apathogenic.

Carry out the test according to general chapter (2.7.17).

Vaccines intended for use in chickens. Administer to each 1-day-old SPF chicken (2.7.7) by the intramuscular route a quantity of the vaccine virus that will allow recovery of virus for the passages described below.

Vaccines intended for use only in chicken embryos or intended for use in chickens and in chicken embryos. Administer to each embryonated egg not later than the recommended day for vaccination by the in ovo route, using the recommended method, a quantity of the vaccine virus that will allow recovery of virus for the passages described below. If the properties of the vaccine virus allow sequential passage through 5 groups via natural spreading, this method may be used, otherwise passage as described below is carried out. 5-7 days after administering the vaccine to chickens or 5-7 days after hatching when the vaccine has been administered in ovo, prepare a suspension of white blood cells from each chicken and pool these samples. Administer a suitable volume of the pooled samples by the intraperitoneal route to each 1-day-old SPF chicken) (2.7.7) of the next group. Carry out this passage operation not fewer than 4 times; verify the presence of the virus at each passage. If the virus is not found at a passage level, repeat the passage by administration to a group of 10 chickens. Carry out the test for residual pathogenicity using the material used for the 1st passage and the virus at the final passage level. Administer the virus by the route to be recommended for

vaccination that is likely to be the least safe for use in these chickens or chicken embryos. The vaccine virus complies with the test if no indication of increase in virulence of the virus recovered for the final passage compared with the material used for the

1st passage is observed. If virus is not recovered after an initial passage in 5 chickens or chicken embryos and a subsequent repeat passage in 10 chickens or chicken embryos, the vaccine virus also complies with the test.

Immunogenicity

A test is carried out for each route and method of administration to be recommended, using in each case chickens of the minimum age to be recommended for vaccination or chicken embryos. The quantity of the vaccine virus administered to each chicken or chicken embryo is not greater than the minimum virus 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.

Vaccines intended for use in chickens. Use not fewer than 60 chickens of the same origin and from an SPF flock (2.7.7). Vaccinate by a route to be recommended not fewer than 30 chickens. Maintain not fewer than 30 chickens as controls.

Vaccines intended for use in chicken embryos. Use embryonated chickens of the same origin and from an SPF flock (2.7.7). Vaccinate by the in ovo route using the method to be recommended, 50 per cent of the embryonated eggs. Maintain 50 per cent of the embryonated eggs as controls. The test is not valid if any group consists of fewer than 30 hatched chicks. Irrespective of whether the vaccine was administered to chickens or chicken embryos, challenge each chicken not later than 9 days after vaccination by a suitable route with a sufficient quantity of virulent Marek's disease virus. Observe the chickens at least daily for 70 days after challenge. Record the deaths and the number of surviving chickens that show clinical signs of disease. At the end of the observation period, euthanise all the surviving chickens and carry out an examination for macroscopic lesions of Marek's disease.

The test is not valid if:

– during the observation period after challenge, fewer than 70 per cent of the control chickens die or show severe clinical signs or macroscopic lesions of Marek's disease;

– and/or, during the period between the vaccination and challenge, more than 10 per cent of the control or vaccinated chickens show abnormal clinical signs or die from causes not attributable to the vaccine.

The vaccine virus complies with the test if the relative protection percentage, calculated using the following expression, is not less than 80 per cent: V- C/100 - C × 100

V = percentage of challenged vaccinated chickens that survive to the end of the observation period without notable clinical signs or macroscopic lesions of Marek's disease;

C = percentage of challenged control chickens that survive to the end of the observation period

without notable clinical signs or macroscopic lesions of Marek's disease.

Batch tests

Identification. Vaccine complies with the requirements of tests mentioned under production. Alternatively suitable validated immunochemical/ molecular biology methods can be used with the approval of competent authority.

Bacterial and fungal contamination (2.2.11). The vaccine complies with the test for sterility (2.2.11). Any diluent supplied with the vaccine shall comply requirements of sterility test as described in 2.2.11.

Mycoplasams (2.7.9). Complies with the test for mycoplasmas.

Water (2.3.43). Not more than 3.0 per cent (For Freeze dried vaccine only

Extraneous agents (2.7.11). The vaccine is free from extraneous agents.

Safety. Use ten one-day-old SPF chickens (2.7.7, Table 3) or healthy susceptible chickens. Administer by recommended route and method to each chicken or chicken embryo 10 doses of the vaccine. Observe the chicken for 21 days. No chicken shows

persistent clinical signs, dies or, at autopsy, shows macroscopic lesions from causes attributable to the vaccine. If during the observation period more than two chickens die from causes not attributable to the vaccine, repeat the test

Note: General Requirements shall be referred regarding omission of the batch safety test.

Virus titre

Vaccine containing one type of virus: Titrate the vaccine virus by inoculation into suitable cell culture derived from SPF eggs (2.7.7). If the virus titre is determined in plaque forming units (PFU), only primary plaques are taken into consideration. The vaccine complies with the test if one dose contains not less than 10^3 PFU per dose.

Vaccine containing more than one type of virus: For vaccine containing more than one type of virus, titrate each virus by inoculation into suitable cell culture derived from SPF eggs (2.7.7), reading the results by immune staining using antibodies. Vaccine complies with the test if one dose contains for each vaccine virus not less than 10^3 PFU of virus per dose.

Potency

Carry out a separate potency test for each of the routes of administration stated on the label. For each of the stated routes, use not less than 30 susceptible one day old SPF chickens (2.7.7) or healthy susceptible chickens.

Administer each chicken a volume of the vaccine containing a quantity of the virus equivalent to minimum titre stated on the label. Use 30 chickens of the same flock and age as controls. After 9 days, challenge each chicken by a suitable route with a suitable quantity of virulent Marek's disease virus. Observe the birds for 10 weeks. Record the deaths and kill the survivors to carry out autopsies on both dead and sacrificed chickens for specific microscopic lesions of Marek's disease for each of the stated routes of administration, the total number of vaccinated birds that show specific macroscopic lesions is reduced by not less than 80% as compared with the control birds and the challenge virus produces specific macroscopic lesions in not less than 70% of the control birds.

If the potency test has been performed with satisfactory results on representative batch of the vaccine from the same seed lot, it may be omitted as routine control test during production of other batches of the vaccine prepared from the same seed lot.

Labelling

The frozen vaccine has to be dispensed in glass ampoules suitable for liquid nitrogen storage and if the below information cannot be printed on the small size ampoule, the product should be accompanied by suitable insert which clarifies the prescribed contents of the labels.

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 or reconstituted with the diluent supplied for reconstitution where applicable"; (4) the animal species for which the vaccine is intended; (5) storage temperatures; (6) Batch Number, Manufacturing date and expiry date; (7) Precautions in pregnant animals (If applicable); (8) Total volume or number of doses; (9) Minimum virus titre per dose of vaccine; (10) Dose of vaccine