Edition: BP 2025 (Ph. Eur. 11.6 update)

Laryngotracheitis Vaccine, Living

General Notices

(Avian Infectious Laryngotracheitis Vaccine (Live), Ph. Eur. monograph 1068)

Ph Eur

1 DEFINITION

Avian infectious laryngotracheitis vaccine (live) is a preparation of a suitable strain of avian infectious laryngotracheitis virus (gallid herpesvirus 1). This monograph applies to vaccines intended for administration to chickens 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 the production of vaccines for veterinary use (*5.2.4*).

2-3 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 chickens for which it is intended.

The following tests for index of respiratory virulence (section 2-3-1), safety (section 2-3-2), increase in virulence (section 2-3-3) and immunogenicity (section 2-3-4) may be used during the demonstration of safety and efficacy.

2-3-1 Index of respiratory virulence

Use for the test not fewer than sixty 10-day-old chickens from an SPF flock (5.2.2). Divide them randomly into 3 groups, maintained separately. Prepare 2 tenfold serial dilutions starting from a suspension of the vaccine virus having a titre of 10^5 EID_{50} or 10^5 $CCID_{50}$ per 0.2 mL or, if not possible, having the maximum attainable titre. Use vaccine virus at the least attenuated passage level that will be present between the master seed lot and a batch of the vaccine. Allocate the undiluted virus suspension and the 2 virus dilutions each to a different group of chickens. Administer by the intratracheal route to each chicken 0.2 mL of the virus suspension attributed to its group. Observe the chickens for 10 days after administration and record the number of deaths. The index of respiratory virulence is the total number of deaths in the 3 groups divided by the total number of chickens. The vaccine virus complies with the test if its index of respiratory virulence is not greater than 0.33.

2-3-2 Safety

Carry out the test for each route and method of administration to be recommended for vaccination, using in each case chickens not older than the minimum age to be recommended for vaccination and from an SPF flock (5.2.2). Use vaccine virus at the least attenuated passage level that will be present in a batch of the vaccine. For each test performed in chickens younger than 3 weeks of age, use not fewer than 10 chickens. For each test performed in chickens older than 3 weeks of age, use not fewer than 8 chickens. Administer to each chicken 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 chickens at least daily for at least 14 days.

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

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

2-3-3 Increase in virulence

Carry out the test according to general chapter 5.2.6 using chickens not more than 2 weeks old, from an SPF flock (5.2.2). 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.

Administer to each chicken of the 1st group by eye-drop a quantity of the vaccine virus that will allow recovery of virus for the passages described below. After the period shown to correspond to maximum replication of the virus, prepare a suspension from the mucosae of suitable parts of the respiratory tract of each chicken and pool these samples. Administer 0.05 mL of the pooled samples by eye-drop to each 2 week-old SPF chicken (5.2.2) 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. Determine the index of respiratory virulence (section 2-3-1) using the material used for the 1st passage and the virus at the final passage level; if the titre of the final passaged virus is less than 10^5 EID_{50} or 10^5 $CCID_{50}$, prepare the tenfold, serial dilutions using the highest titre available.

The vaccine virus complies with the test if no indication of an 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 and a subsequent repeat passage in 10 chickens, the vaccine virus also complies with the test.

2-3-4 Immunogenicity

A test is carried out for each route and method of administration to be recommended for vaccination using in each case chickens not older than the minimum age to be recommended for vaccination. The quantity of the vaccine virus administered to each chicken 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. Use for the test not fewer than 30 chickens of the same origin and from an SPF flock (5.2.2). Vaccinate by a route to be recommended not fewer than 20 chickens. Maintain not fewer than 10 chickens as controls. Challenge each chicken after 21 days by the intratracheal route with a sufficient quantity of virulent infectious laryngotracheitis virus. Observe the chickens at least daily for 7 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 examination for macroscopic lesions: mucoid, haemorrhagic and pseudomembraneous inflammation of the trachea and orbital sinuses.

The test is not valid if:

— during the observation period after challenge fewer than 90 per cent of the control chickens die or show severe clinical signs of avian infectious laryngotracheitis or notable macroscopic lesions of the trachea and orbital sinuses;

— or if during the period between the vaccination and challenge more than 10 per cent of the vaccinated or control chickens show notable clinical signs of disease 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 disease and/or macroscopical lesions of the trachea and orbital sinuses.

3 BATCH TESTS

3-1 Identification

The vaccine, diluted if necessary, is identified using a suitable method. For example, when mixed with a monospecific infectious laryngotracheitis virus antiserum, it is no longer able to infect embryonated hens' eggs from an SPF flock (*5.2.2*) or susceptible cell cultures (*5.2.4*) into which it is inoculated.

3-2 Bacteria and fungi

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

Any diluent supplied for reconstitution of the vaccine complies with the test for sterility prescribed in the general monograph *Vaccines for veterinary use (0062)*.

3-3 Mycoplasmas (*2.6.7*)

The vaccine complies with the test for mycoplasmas.

3-4 Extraneous agents (*5.2.5*)

The vaccine is free from extraneous agents.

3-5 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 titre stated on the label.

3-6 Potency

The vaccine complies with the requirements of the test prescribed under Immunogenicity (section 2-3-4) when administered according to the recommended schedule by a recommended route and method. 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.