AVIAN INFECTIONOUS LARYNGOTRACHEITIS VACCINE (LIVE)

Vaccinum laryngotracheitidis infective aviairie vivum

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 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 chickens for which it is intended.

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

2.4-1. 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 $10^6$ EID$_{50}$ or $10^6$ 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 in 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 attributable 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.

2-4-2. 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 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 each test use not fewer than 20 chickens, from an SPF flock (5.2.2). 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 21 days. The test is not valid if more than 10 per cent of the chickens die from causes not attributable to the vaccine virus. The vaccine virus complies with the test if no chicken shows notable clinical signs of avian infectious laryngotracheitis or dies from causes attributable to the vaccine virus.

2-4-3. 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 chickens not more than 2 weeks old, from an SPF flock (5.2.2), 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 passed virus that has been recovered is tested for increase in virulence. Care must be taken to avoid contamination by virus from previous passages. Administer 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 of 5 other chickens that are 2 weeks old and from an SPF flock (5.2.2). 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. Determine the index of respiratory virulence (section 2-4-1) using the unpassaged vaccine virus and the maximally passed virus that has been recovered; if the titre of the maximally 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 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.

2.4-4. Immunogenicity. A test is carried out for each route and method of administration to be recommended using in each case chickens not older than the youngest 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 recommended route 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 kill all the surviving chickens and carry out examination for macroscopic lesions: pseudomembranous inflammation of the trachea and orbital sinuses. The test is not valid if:
Vaccines for veterinary use (0062) with the test for sterility prescribed in the monograph Vaccines intended for administration by injection comply Vaccines not intended for administration by injection Bacteria and fungi 3-2. an SPF flock (mixed with a monospecific infectious laryngotracheitis virus 728 3-1. Identification 3. BATCH TESTS 5.2.2 Potency 5.2.6 PRODUCTION The vaccine is propagated in embryonated eggs from healthy flocks or in suitable cell cultures (5.2.4). The test for inactivation is carried out in embryonated eggs or suitable cell cultures and the quantity of inactivated virus used is equivalent to not less than ten doses of vaccine. No live virus is detected. The vaccine may contain an adjuvant. CHOICE OF VACCINE COMPOSITION The vaccine is shown to be satisfactory with respect to safety (5.2.6) and immunogenicity (5.2.7) for each category of turkeys for which it is intended. The following test may be used during demonstration of immunogenicity. Immunogenicity. The test described under Potency is suitable for demonstrating immunogenicity. BATCH TESTING Batch potency test. Carry out a suitable validated test for which satisfactory correlation with the test described under Potency has been established, the criteria for acceptance being set with reference to a batch that has given satisfactory results in the latter test. IDENTIFICATION When injected into animals free from antibodies against avian paramyxovirus 3, the vaccine stimulates the production of such antibodies. TESTS Safety. Inject twice the vaccinating dose by a recommended route into each of ten turkeys, 14 to 28 days old and free from antibodies against avian paramyxovirus 3. Observe the birds for 21 days. No abnormal local or systemic reaction occurs. Inactivation. Inject two-fifths of a dose into the allantoic cavity of each of ten embryonated hen eggs, 9 to 11 days old, from flocks free from specified pathogens (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 avian paramyxovirus 3: the vaccine does not comply with the test if avian paramyxovirus 3 is found. Inject into the allantoic cavity of each of ten SPF eggs, 9 to 11 days old, 0.2 ml of the pooled allantoic fluid from the live embryos and, into each of ten similar eggs, 0.2 ml of the pooled fluid from the dead embryos and incubate for 5 to 6 days. Test the allantoic fluid from each egg for the presence of haemagglutinins using chicken erythrocytes.