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

Aujeszky's Disease Vaccine, Living

General Notices

(Aujeszky's Disease Vaccine (Live) for Pigs for Parenteral Administration, Ph. Eur. monograph 0745)

Ph Eur

1 DEFINITION

Aujeszky's disease vaccine (live) for pigs for parenteral administration is a preparation of a suitable strain of Aujeszky's disease virus. This monograph applies to vaccines intended for the active immunisation of pigs and for the passive protection of their progeny against Aujeszky's disease. The vaccine may be administered after mixing with an adjuvant.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The vaccine virus is grown in cell cultures.

2-2 SUBSTRATE FOR VIRUS PROPAGATION

2-2-1 Cell cultures

The cell cultures 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 is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*) for the pigs for which it is intended. The virus may have a genetic marker.

The following tests for safety (section 2-3-1), virus excretion (section 2-3-2), non-transmissibility, including transmission across the placenta and by semen (section 2-3-3), increase in virulence (section 2-3-4) and immunogenicity (section 2-3-5), may be used during the demonstration of safety and efficacy.

2-3-1 Safety

2-3-1-1 Safety test in piglets. Carry out the test for each route and method of administration to be recommended for vaccination, using in each case piglets 3-4 weeks old. 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 piglets that do not have antibodies against Aujeszky's disease virus. Administer to not fewer than 10 piglets 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. Maintain not fewer than 10 piglets as controls. Observe the piglets at least daily for at least 21 days.

The vaccine virus complies with the test if the weight curve of the vaccinated piglets does not differ significantly from that of the controls and if no piglet shows signs of disease or dies from causes attributable to the vaccine virus.

2-3-1-2 Safety of the pigs used in tests 2-3-5 for immunogenicity. The pigs used in the tests for immunogenicity are also used to evaluate safety. Measure the body temperature of each vaccinated pig at the time of vaccination and 6 h, 24 h and 48 h later. Examine the injection site at slaughter for local reactions.

The vaccine virus complies with the test if no pig shows:

— a temperature rise greater than 1.5 °C and the number of pigs showing a temperature greater than 41 °C does not exceed 10 per cent of the group;

— other systemic reactions (for example, anorexia);

— abnormal local reactions attributable to the vaccine virus.

2-3-1-3 Safety in field studies. The pigs used for field trials are also used to evaluate safety. Carry out a test in each category of pigs for which the vaccine is intended (sows, fattening pigs). Use not fewer than 3 groups, each of not fewer than 20 pigs, with corresponding groups of not fewer than 10 controls. Measure the body temperature of each pig at the time of vaccination and 6 h, 24 h and 48 h later. Examine the injection site at slaughter for local reactions.

The vaccine virus complies with the test if no pig shows:

— a temperature rise greater than 1.5 °C and the number of pigs showing a temperature greater than 41 °C does not exceed 25 per cent of the group;

— abnormal local reactions attributable to the vaccine virus.

2-3-1-4 Neurological safety. Use for the test not fewer than 10 piglets, 3-5 days old and that do not have antibodies against Aujeszky's disease virus. Administer to each piglet by the intranasal route 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 piglets at least daily for at least 21 days.

The vaccine virus complies with the test if none of the piglets dies or shows signs of neurological disorder attributable to the vaccine virus.

2-3-1-5 Neurological safety for strains other than gE-negative. This test is not necessary for gE-negative strains. Administer to not fewer than 5 piglets, 3-5 days old, by the intracerebral route, $10^{4.5}$ $CCID_{50}$ of vaccine virus.

The vaccine virus complies with the test if none of the piglets dies or shows signs of neurological disorder.

2-3-1-6 Absence of latent infections. Use for the test not fewer than 10 piglets, 3-4 weeks old and that do not have antibodies against Aujeszky's disease virus. Administer to each piglet a daily injection of 2 mg of prednisolone per kilogram of body mass for 5 consecutive days. On the 3rd day administer to each piglet a quantity of vaccine virus equivalent to not less than the maximum virus titre likely to be contained in 1 dose of the vaccine by a route to be recommended. Antimicrobial agents may be administered to prevent aspecific signs. Observe the piglets at least daily for at least 21 days.

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

2-3-1-7 Safety in pregnant sows and absence of transmission across the placenta. Use for the test not fewer than 15 pregnant sows that do not have antibodies against Aujeszky's disease virus. Administer to not fewer than 5 sows, by a route to be recommended, a quantity of vaccine virus equivalent to not less than 10 times the maximum virus titre likely to be contained in 1 dose of the vaccine during the 4th or 5th week of gestation. Administer to not fewer than 5 other sows the same dose of the virus by the same route during the 10th or 11th weeks of gestation. Maintain not fewer than 5 other pregnant sows as controls. For the piglets from vaccinated sows: carry out tests for serum antibodies against Aujeszky's disease virus; carry out tests for Aujeszky's disease virus antigen in the liver and lungs of those piglets showing abnormalities and in a quarter of the remaining healthy piglets.

The vaccine virus complies with the test if:

— the number of piglets born to the vaccinated sows, any abnormalities in the piglets and the duration of gestation do not differ significantly from those of the controls;

— no Aujeszky's disease virus antigen is found in piglets born to the vaccinated sows;

— no antibodies against Aujeszky's disease virus are found in the serum taken before ingestion of the colostrum.

2-3-2 Virus excretion

Use for the test not fewer than 18 pigs, 3-4 weeks old and that do not have antibodies against Aujeszky's disease virus. Administer to not fewer than 14 pigs a quantity of the vaccine virus equivalent to not less than the maximum virus titre likely to be contained in 1 dose of the vaccine by a route and a site to be recommended. Maintain not fewer than 4 pigs as contact controls. Carry out suitably sensitive tests for the virus individually on the nasal and oral secretions as follows: collect nasal and oral swabs daily from the day before vaccination until 10 days after vaccination.

The vaccine complies with the test if the virus is not isolated from the secretions collected.

2-3-3 Non-transmissibility

Carry out the test on 4 separate occasions. Each time, administer to not fewer than 4 piglets, 3-4 weeks old and that do not have antibodies against Aujeszky's disease virus, by a route to be recommended, a quantity of the vaccine virus equivalent to not less than the maximum virus titre likely to be contained in 1 dose of the vaccine. After 1 day, keep not fewer than 2 other piglets of the same age and that do not have antibodies against Aujeszky's disease virus close together with them. After 5 weeks, test all the piglets for the presence of antibodies against Aujeszky's disease virus.

The test is not valid if any vaccinated piglet does not show an antibody response. The vaccine virus complies with the test if no antibodies against Aujeszky's disease virus are detected in any group of contact controls and if all the vaccinated piglets show an antibody response.

2-3-4 Increase in virulence

Carry out the test according to general chapter *5.2.6* using piglets 3-5 days old and that do not have antibodies against Aujeszky's disease virus. 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 piglet of the 1st group by the intranasal route a quantity of the vaccine virus that will allow recovery of virus for the passages described below. After 3-5 days, prepare a suspension from the brain, lung, tonsils and local lymph glands of each piglet and pool the samples. Administer 1 mL of the suspension of pooled samples by the intranasal route to each piglet 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 piglets.

If the 5th group of piglets shows no evidence of an increase in virulence indicative of reversion during the observation period, further testing is not required. Otherwise, carry out an additional safety test and compare the clinical signs and any relevant parameters in a group of at least 8 piglets receiving the material used for the 1st passage and another similar group receiving the virus at the final passage level.

The vaccine virus complies with the test if no indication of increased 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 2 piglets and a subsequent repeat passage in 10 piglets, the vaccine virus also complies with the test.

2-3-5 Immunogenicity

A test is carried out for each route and method of administration to be recommended for vaccination. The quantity of vaccine virus to be administered to each pig 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 vaccine.

2-3-5-1 Vaccines intended for active immunisation. Use for the test not fewer than 15 fattening pigs of the age to be recommended and that do not have antibodies against Aujeszky's disease virus. The body mass of none of the pigs differs from the average body mass of the group by more than 20 per cent. Vaccinate not fewer than 10 pigs, according to the schedule to be recommended. Maintain not fewer than 5 pigs as controls. At the end of the fattening period (80-90 kg), weigh and challenge each pig by the intranasal route with a sufficient quantity of virulent Aujeszky's disease virus (challenge with at least 10^6 $CCID_{50}$ of a virulent strain having undergone not more than 3 passages and administered in not less than 4 mL of diluent has been found to be satisfactory). Determine the titre of virus in swabs taken from the nasal cavity of each pig daily from the day before challenge until virus is no longer detected. Weigh each pig 7 days after challenge or at the time of death if this occurs earlier and calculate the average daily gain as a percentage. For each group (vaccinated and controls), calculate the average of the average daily gains.

The test is invalid unless all the control pigs display signs of Aujeszky's disease and the average of their average daily gains is less than -0.5 kg. The vaccine complies with the test if:

— all the vaccinated pigs survive and the difference between the averages of the average daily gains for the 2 groups is not less than 1.5 kg;

— the geometrical mean titres and the duration of excretion of the challenge virus are significantly lower in vaccinates than in controls.

2-3-5-2 Vaccines intended for passive protection. If the vaccine is intended for use in sows for the passive protection of piglets, the suitability of the vaccine virus for this purpose may be demonstrated by the following method.

Use for the test not fewer than 12 sows that do not have antibodies against Aujeszky's disease virus. Vaccinate not fewer than 8 sows, according to the schedule to be recommended. Maintain not fewer than 4 sows as controls. At 6-10 days of age, challenge the piglets from the sows with a sufficient quantity of virulent Aujeszky's disease virus. Observe the piglets at least daily for 21 days.

The test is not valid if the average number of piglets per litter for each group is less than 6.

The vaccine complies with the test if not less than 80 per cent protection against mortality is found in the piglets from the vaccinated sows compared to those from the control sows.

2-4 MANUFACTURER'S TESTS

2-4-1 Batch potency test

It is not necessary to carry out the Potency test (section 3-6) 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. The test described under Potency is carried out for a given vaccine, on one or more occasions, as decided by or with the agreement of the competent authority. Where this 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.

3 BATCH TESTS

3-1 Identification

The vaccine virus is identified using a suitable method. For example, when mixed with a monospecific antiserum, the vaccine virus is no longer able to infect susceptible cell cultures into which it is inoculated.

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 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 in suitable cell cultures. The vaccine complies with the test if 1 dose contains not less than the minimum virus titre stated on the label.

3-6 Potency

The vaccine complies with the requirements of the test described below when administered by a recommended route and method.

Use not fewer than 10 pigs weighing 15-35 kg and that do not have antibodies against Aujeszky's disease virus. The body mass of none of the pigs differs from the average body mass of the group by more than 25 per cent. Vaccinate not fewer than 5 pigs with 1 dose of the vaccine. Maintain not fewer than 5 pigs as controls. After 3 weeks, weigh each pig, then challenge them by the intranasal route with a sufficient quantity of virulent Aujeszky's disease virus. Weigh each pig 7 days after challenge or at the time of death if this occurs earlier and calculate the average daily gain as a percentage. For each group (vaccinated and controls), calculate the average of the average daily gains.

The test is invalid unless all the control pigs display signs of Aujeszky's disease and the average of their average daily gains is less than -0.5 kg. The vaccine complies with the test if all the vaccinated pigs survive and the difference between the averages of the average daily gains for the 2 groups is not less than 1.6 kg.

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