AUJESZKY’S DISEASE VACCINE (LIVE) FOR PIGS FOR PARENTERAL ADMINISTRATION, FREEZE-DRIED

Vaccinum morbi Aujeszkyi ad suem vivum cryodesicatum ad usum parenterale

DEFINITION

Freeze-dried Aujeszky’s disease vaccine (live) for pigs for parenteral administration is a preparation of an attenuated strain of Aujeszky’s disease virus. It may be administered after mixing with an adjuvant.

PRODUCTION

The virus strain is grown in suitable cell cultures (5.2.4) or in fertilised hen eggs from flocks free from specified pathogens (5.2.2). The viral suspension is harvested, mixed with a suitable stabilising liquid and freeze-dried.

CHOICE OF VACCINE STRAIN

Only a virus strain shown to be satisfactory with respect to the following characteristics may be used in the preparation of the vaccine: safety; transmissibility, including transmission across the placenta and by semen; irreversibility of attenuation; immunogenicity. The strain may have a genetic marker. The following tests may be used during demonstration of safety (5.2.6) and efficacy (5.2.7).

Safety.

A. 10 piglets, 3 to 4 weeks old and which do not have antibodies against Aujeszky’s disease virus or against a fraction of the virus, each receive by a recommended route a quantity of virus corresponding to 10 doses of vaccine. 10 piglets of the same origin and age which do not have antibodies against Aujeszky’s disease virus or against a fraction of the virus are kept as controls. The animals are observed for 21 days. The piglets remain in good health.

B. The animals used in the test for immunogenicity are also used to evaluate safety. The rectal temperature of each vaccinated animal is measured at the time of vaccination and 6 h, 24 h and 48 h later. No animal shows a temperature rise greater than 1.5 °C and the number of animals showing a temperature greater than 41 °C does not exceed 10 per cent of the group. No other systemic reactions (for example, anorexia) are noted. At slaughter, the injection site is examined for local reactions. No abnormal local reactions attributable to the vaccine are produced.

C. The animals used for field trials are also used to evaluate safety. A test is carried out in each category of animals for which the vaccine is intended (sows, fattening pigs). Not fewer than 3 groups each of not fewer than 20 animals are used with corresponding groups of not fewer than 10 controls. The rectal temperature of each animal is measured at the time of vaccination and 6 h, 24 h and 48 h later. No animal shows a temperature rise greater than 1.5 °C and the number of animals showing a temperature greater than 41 °C does not exceed 25 per cent of the group. At slaughter, the injection site is examined for local reactions. No abnormal local reactions attributable to the vaccine are produced.

D. 10 piglets, 3 to 5 days old and which do not have antibodies against Aujeszky’s disease virus or against a fraction of the virus, each receive by the intranasal route a quantity of virus corresponding to 10 doses of vaccine. The animals are observed for 21 days. None of the piglets dies or shows signs of neurological disorder attributable to the vaccine virus.

E. This test is not necessary for gE-negative strains. 5 piglets, 3 to 5 days old, each receive $10^{9.3}$ CCID$_{50}$ of vaccine virus intracerebrally. None of the piglets dies or shows signs of neurological disorder.

F. 10 piglets, 3 to 4 weeks old and which do not have antibodies against Aujeszky’s disease virus or against a fraction of the virus, each receive a daily injection of 2 mg of prednisolone per kilogram of body mass for 5 consecutive days. On the third day each piglet receives a quantity of virus corresponding to 1 dose of vaccine by a recommended route. Antimicrobial agents may be administered to prevent aspecific signs. Observe the animals for 21 days following administration of the virus. The piglets remain in good health.

G. 15 pregnant sows which do not have antibodies against Aujeszky’s disease virus or against a fraction of the virus are used. Each of 5 sows receive by a recommended route a quantity of virus corresponding to 10 doses of vaccine during the fourth or fifth week of gestation. 5 other sows each receive the same dose of virus by the same route during the tenth or eleventh week of gestation. The other 5 pregnant sows are kept as controls. 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. 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. No Aujeszky’s disease virus antigen is found in piglets born to the vaccinated sows and no antibodies against Aujeszky’s disease virus are found in the serum taken before ingestion of colostrum.

Virus excretion. 18 pigs, 3 to 4 weeks old and which do not have antibodies against Aujeszky’s disease virus or against a fraction of the virus are used. 14 of the pigs each receive 1 dose of vaccine by the recommended route and at the recommended site and the remaining 4 pigs are kept as contact controls. Suitably sensitive tests for the virus are carried out individually on the nasal and oral secretions as follows: nasal and oral swabs are collected daily from the day before vaccination until 10 days after vaccination. The vaccine is acceptable if the virus is not isolated from the secretions collected.

Transmissibility. The test is carried out on 4 separate occasions. Each time 4 piglets, 3 to 4 weeks old and which do not have antibodies against Aujeszky’s disease virus or against a fraction of the virus, each receive by a recommended route a quantity of virus corresponding to 1 dose of vaccine. 1 day after the administration, 2 other piglets of the same age which do not have antibodies against Aujeszky’s disease virus or against a fraction of the virus are kept close together with them. After 5 weeks all the animals are tested for the presence of antibodies against Aujeszky’s disease virus. Antibodies against Aujeszky’s disease virus are not detected in any group of contact controls. All the treated piglets show an antibody response.

Reversion to virulence. 2 piglets, 3 to 5 days old and which do not have antibodies against Aujeszky’s disease virus or against a fraction of the virus, each receive by the intranasal route a quantity of virus corresponding to 1 dose of vaccine. 3 to 5 days later brain, lung, tonsils and local lymph glands are taken from each piglet and the samples are pooled. 1 ml of the pooled organ suspension is administered...
intranasally into each of 2 other piglets of the same age and susceptibility. This operation is then repeated not fewer than 4 times, the last time in not fewer than 5 piglets. The presence of the virus is verified at each passage by direct or indirect means. If the virus has disappeared, a second series of passages is carried out. The piglets do not die or show neurological disorders from causes attributable to the vaccine virus. There is no indication of an increase of virulence as compared with the non-passaged virus.

**Immunogenicity.** Not fewer than 10 fattening pigs of the age recommended for vaccination and which do not have antibodies against Aujeszky’s disease virus or against a fraction of the virus are vaccinated according to the recommended schedule and by a recommended route. 5 similar pigs are used as controls. At the end of the fattening period (80 kg to 90 kg), each pig is weighed and then challenged by the intranasal route with a suitable quantity of a virulent strain of Aujeszky’s disease virus (challenge with at least 10^6 CCID₅₀ 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). The titre of virus is determined in swabs taken from the nasal cavity of each pig daily from the day before challenge until virus is no longer detected. Each pig is weighed 7 days after challenge or at the time of death if this occurs earlier and the average daily gain is calculated as a percentage. For each group (vaccinated and controls), the average of the average daily gains is calculated. 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,
- the geometrical mean titres and the duration of excretion of the challenge virus are significantly lower in vaccinates than in controls.

The test is not valid unless all the control pigs display signs of Aujeszky’s disease and the average of their average daily gains is less than ~0.5.

If the vaccine is intended for use in sows for the passive protection of piglets, the suitability of the strain for this purpose may be demonstrated by the following method. 8 sows which do not have antibodies against Aujeszky’s disease virus or against a fraction of the virus are vaccinated according to the recommended schedule and by the recommended route; 4 sows are kept as controls. The piglets from the sows are challenged with a suitable quantity of a virulent strain of Aujeszky’s disease virus at 6 to 10 days of age. The piglets are observed for 21 days. The vaccine is satisfactory 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. The test is not valid if the average number of piglets per litter for each group is less than 6.

**BATCH TESTING**

The test described under Potency is not necessarily carried out for routine testing of batches of vaccine. It is carried out for a given vaccine, on one or more occasions, as decided by or with the agreement of the competent authority; where the test is not carried out a suitable, validated alternative test is carried out, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency.

**IDENTIFICATION**

In animals having no antibodies against Aujeszky’s disease virus or against a fraction of the virus, the vaccine stimulates the production of specific neutralising antibodies.