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Furunculosis Vaccine for Salmonids, Inactivated

General Notices

(Furunculosis Vaccine (Inactivated, Oil-Adjuvanted, Injectable) for Salmonids, Ph. Eur. monograph 1521)

Ph Eur

1 DEFINITION

Furunculosis vaccine (inactivated, oil-adjuvanted, injectable) for salmonids is prepared from cultures of one or more suitable strains of *Aeromonas salmonicida* subsp. *salmonicida*, inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of salmonids against furunculosis.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The strains of *A. salmonicida* are cultured and harvested separately. The harvests are inactivated by a suitable method. They may be purified and concentrated. Whole or disrupted cells may be used and the vaccine may contain extracellular products of the bacterium released into the growth medium. The vaccine contains an oily adjuvant.

2-2 CHOICE OF VACCINE STRAIN

The strains included in the vaccine are shown to be suitable with respect to the production of antigens of assumed protective importance. The vaccine is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*) in the species of fish for which it is intended.

The following tests for safety (section 2-2-1) and immunogenicity (section 2-2-2) may be used during the demonstration of safety and efficacy.

2-2-1 Safety

2-2-1-1 Laboratory test. Carry out the test in each species of fish for which the vaccine is intended, using fish of the minimum body mass to be recommended for vaccination. Use a batch of vaccine containing not less than the maximum potency that may be expected in a batch of vaccine.

Use not fewer than 50 fish from a population that does not have specific antibodies against *A. salmonicida* subsp. *salmonicida* and has not been vaccinated against or exposed to furunculosis. The test is carried out in the conditions to be recommended for the use of the vaccine with a water temperature not less than 10 °C. Administer to each fish by the intraperitoneal route 1 dose of the vaccine. Observe the fish at least daily for 21 days.

The test is not valid if more than 6 per cent of the fish die from causes not attributable to the vaccine. The vaccine complies with the test if no fish shows abnormal local or systemic reactions or dies from causes attributable to the vaccine.

2-2-1-2 Field studies. Safety is also demonstrated in field trials by administering the intended dose to a sufficient number of fish in not fewer than 2 sets of premises. Samples of 30 fish are taken on 3 occasions (after vaccination, at the middle of

the rearing period and at slaughter) and examined for local reactions in the body cavity. Moderate lesions involving localised adhesions between viscera or between viscera and the abdominal wall and slight opaqueness and/or sparse pigmentation of the peritoneum are acceptable. Extensive lesions including adhesions between greater parts of the abdominal organs, massive pigmentation and/or obvious thickening and opaqueness of greater areas of the peritoneum are unacceptable if they occur in more than 10 per cent of the fish in any sample. Such lesions include adhesions that give the viscera a ‘one-unit' appearance and/or lead to manifest laceration of the peritoneum following evisceration.

2-2-2 Immunogenicity

Carry out the test according to a protocol defining limits of body mass for the fish, water source, water flow and temperature limits, and preparation of a standardised challenge. A test is carried out for the route and method of administration to be recommended. The vaccine administered to each fish is of minimum potency.

Use for the test not fewer than 60 fish from a population that does not have specific antibodies against *A. salmonicida* subsp. *salmonicida* and has not been vaccinated against or exposed to furunculosis. Vaccinate not fewer than 30 fish according to the instructions for use. Perform mock vaccination on a control group of not fewer than 30 fish; mark vaccinated and control fish for identification. Keep all the fish in the same tank or mix equal numbers of controls and vaccinates in each tank if more than one tank is used. Where justified and when fish cannot be marked, non-marked fish may be used. Vaccinates and controls may then be kept in the same tank but physically separated (for example by fishing nets). Challenge each fish, by injection, at a fixed interval after vaccination corresponding to the onset of immunity claimed, with a sufficient quantity of a culture of *A. salmonicida* subsp. *salmonicida* whose virulence has been verified. Observe the fish at least daily until the end of mortality is reached in the control group (no fish have died over a period of 2 days).

The test is not valid if the specific mortality is less than 60 per cent in the control group 21 days after the 1st death in the fish. Calculate the relative percentage survival (RPS) using the following expression:

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V	=	percentage of mortality in vaccinates;
C	=	percentage of mortality in controls.

The vaccine complies with the test if the RPS is not less than 70 per cent.

2-3 MANUFACTURER'S TESTS

2-3-1 Batch potency test

The potency test (section 3-3) may be carried out for each batch of vaccine, using fish of one of the species for which the vaccine is intended. Where the test is not carried out, an alternative validated method may be 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. The following test may be used.

Use not fewer than 35 fish from a population that does not have specific antibodies against *A. salmonicida* subsp. *salmonicida* and that are within specified limits for body mass. Carry out the test at a defined temperature not less than 12 °C. Inject intraperitoneally into each of not fewer than 25 fish 1 dose of vaccine, according to the instructions for use. Perform mock vaccination on a control group of not fewer than 10 fish. Collect blood samples from vaccinates and controls at a defined time not less than 500 degree days after vaccination. Determine for each sample the level of specific antibodies against *A. salmonicida* subsp. *salmonicida* by a suitable immunochemical method (*2.7.1*).

The test is not valid if the control group shows antibodies against *A. salmonicida* subsp. *salmonicida*.

The vaccine complies with the test if the mean level of antibodies in the vaccinates is not significantly lower than that found for a batch that gave satisfactory results in the test described under Potency.

3 BATCH TESTS

3-1 Identification

The vaccine contains the antigen or antigens stated under Definition.

3-2 Bacteria and fungi

The vaccine, including where applicable the diluent supplied for reconstitution, complies with the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)*.

3-3 Potency

The vaccine complies with the requirements of the test mentioned under Immunogenicity (section 2-2-2) when administered by the recommended route and method.

4 LABELLING

The label states information on the time needed for development of immunity after vaccination under the range of conditions corresponding to the recommended use.