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Vibriosis Vaccine for Sea Bass, Inactivated

General Notices

(Vibriosis Vaccine (Inactivated) for Sea Bass, Ph. Eur. monograph 3090)

Ph Eur

1 DEFINITION

Vibriosis vaccine (inactivated) for sea bass is prepared from cultures of one or more suitable strains or serovars of *Vibrio (Listonella) anguillarum*, inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for administration by injection or immersion for the active immunisation of sea bass against vibriosis.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The strains of *V. anguillarum* 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 bacteria released into the growth medium. The vaccine may be adjuvanted.

2-2 CHOICE OF VACCINE COMPOSITION

The strains of *V. anguillarum* used are shown to be suitable with respect to 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 sea bass.

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 tests. Safety is tested using test 2-2-1-1-1, test 2-2-1-1-2, or both, depending on the recommendations for use.

Carry out the test using sea bass 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. The test is carried out in the conditions to be recommended for use of the vaccine with a water temperature not less than 19 °C.

2-2-1-1-1 Vaccines intended for administration by injection. Use not fewer than 50 fish from a population that has not been vaccinated against or exposed to vibriosis. 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.

2-2-1-1-2 Vaccines intended for administration by immersion. Use not fewer than 50 fish from a population that has not been vaccinated against or exposed to vibriosis. Prepare an immersion bath at twice the concentration to be recommended. Bathe the fish for twice the time to be recommended. 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 Vaccines intended for administration by injection: long-term safety studies. The development of lesions is examined in longer term studies where the fish are representative of a commercial weight of at least 200 g. Samples of a sufficient number of fish in not fewer than 2 sets of premises are taken on up to 3 occasions including after vaccination and at slaughter. The post-mortem examination includes examination of local reactions in the abdominal cavity and evaluation of the visual appearance of the abdominal cavity and a scoring of the severity of the lesions according to an established scoring system. Moderate adhesions connecting organs to the abdominal wall with minor visible lesions after evisceration are acceptable. The presence of vesicles including cystic brown vesicles less than 1 mm in diameter in adipose tissue is also acceptable. Major adhesions with granuloma connecting all the internal organs with more severe lesions that are hard to remove manually are unacceptable if they occur in more than 10 per cent of the fish in any sample.

2-2-2 Immunogenicity

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

Use for the test not fewer than 60 fish of the body mass to be recommended for vaccination, from a population that has not been vaccinated against or exposed to vibriosis. 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 1 tank is used. Where justified and when fish cannot be marked, non-marked fish may be used. Vaccinates and controls should then be kept in the same tank but physically separated (for example by fishing nets). Challenge each fish at a fixed interval after vaccination, corresponding to the onset of immunity claimed, by a suitable route with a sufficient quantity of cultures of *V. anguillarum* whose virulence has been verified. Observe the fish at least daily until at least 60 per cent specific mortality is reached in the control group. Plot for both vaccinates and controls a curve of specific mortality against time from challenge and determine by interpolation the time corresponding to 60 per cent specific mortality in controls.

The test is not valid if the specific mortality is less than 60 per cent in the control group 21 days after the death of the first fish. Read from the curve for vaccinates the mortality (M) at the time corresponding to 60 per cent mortality in controls. Calculate the relative percentage survival (RPS) using the following expression:

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The vaccine complies with the test if the RPS is not less than 60 per cent for vaccines administered by immersion and 75 per cent for vaccines administered by injection.

2-3 MANUFACTURER'S TEST

2-3-1 Batch potency test

The potency test (section 3-3) may be carried out for each batch of vaccine, using sea bass. 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 30 sea bass, from a population that that has not been vaccinated against or exposed to vibriosis, and that are within specified limits for body mass. Carry out the test at a defined temperature. Carry out the vaccination according to the instructions for use. Perform mock vaccination on a control group of not fewer than 10 fish. Challenge each fish at a fixed interval with a suitable quantity of a virulent strain by intraperitoneal route and observe the fish for a period of at least 21 days.

The test is not valid if the specific mortality is less than 60 per cent in the control group 21 days after the death of the first fish. The vaccine complies with the test if the RPS is not less than 60 per cent for vaccines administered by immersion and 75 per cent for vaccines administered by injection in the vaccinated group.

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 diluents 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 for immunogenicity (section 2-2-2) when administered by a recommended route and method.

4 LABELLING

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