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Winter Ulcer Vaccine for Salmonids

General Notices

(Winter Ulcer Vaccine (Inactivated, Oil-Adjuvanted, Injectable) for Salmonids, Ph. Eur. monograph 2151)

Ph Eur

1 DEFINITION

Winter ulcer vaccine (inactivated, oil-adjuvanted, injectable) for salmonids is prepared from cultures of one or more suitable strains of *Moritella viscosa*.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The strains of *M. viscosa* are cultured and harvested separately. The harvests are inactivated by an appropriate method and may be purified and/or 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 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 or maximum antigen content 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 *M. viscosa* and has not been vaccinated against or exposed to *M. viscosa*. The test is carried out under 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 (e.g. 2-3 on the Speilberg scale if used) including localised adhesions between viscera or between viscera and the abdominal wall, slight

opaqueness and, 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, after intraperitoneal administration of the vaccine. The vaccine administered to each fish is of minimum potency or minimum antigen content.

Use for the test not fewer than 60 fish from a population that does not have specific antibodies against *M. viscosa* and have not been vaccinated against winter ulcer or exposed to *M. viscosa*. Vaccinate not fewer than 30 fish according to the instructions for use. The negative control group consists of not fewer than 30 fish. Mark vaccinated and negative control fish for identification. Keep all the fish in the same tank or mix equal numbers of negative 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 the fish by a suitable method at a fixed interval after vaccination corresponding to the claimed onset of immunity, with a sufficient quantity of *M. viscosa* where 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 the controls.

Specific mortality is caused by infection with *M. viscosa*. 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 (RPS60). Calculate the relative percentage survival (RPS) using the following expression:

The vaccine complies with the test if the RPS is not less than 60 per cent.

A lethal endpoint assessment may be replaced by an observation of clinical signs and application of an endpoint earlier than death to reduce animal suffering. Fish showing skin lesions or clinical signs of disease attributable to *M. viscosa* infection, such as superficial lesions that do not permeate the basement membrane, swelling of scale pockets, superficial skin oedema and regenerated lesions, are euthanised and counted as unprotected fish. Record the number of unprotected fish until the end of challenge. These criteria have to be aligned to give a specification equivalent to RPS60.

2-3 MANUFACTURER'S TESTS

2-3-1 Batch potency test

It is not necessary to carry out the potency test (section 3-3) for each batch of vaccine if it has been carried out using a batch of vaccine with a minimum potency. Where the potency test is carried out, efforts to validate the test using humane end points should be made or one of the following tests may be used.

In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, such alternative validated methods should preferably be used for routine testing.

Carry out the test for antigen content (section 2-3-1-1) together with the test for adjuvant (section 2-3-1-2). If the nature of the product does not allow valid results to be obtained with these tests, or if the vaccine does not comply, the test for serological assay (section 2-3-1-3) is carried out. If the vaccine does not comply with the latter test, the test in fish (section 2-2-2) may be carried out.

2-3-1-1 Antigen content. The relative antigen content is determined by comparing the content of *M. viscosa* antigen per dose of vaccine with a batch of vaccine that has given satisfactory results in the test described under Potency. Before estimation, the antigen may be extracted from the emulsion using a suitable method. The vaccine complies with the test if the estimated antigen content is not significantly lower than that of a batch that has been found to be satisfactory with respect to immunogenicity (section 2-2-2).

2-3-1-2 Adjuvant. The adjuvant is tested by suitable physical and chemical methods. For oil-adjuvanted vaccines, the adjuvant is tested in accordance with the monograph *Vaccines for veterinary use (0062)*. If the adjuvant cannot be

adequately characterised, the antigen content determination cannot be used as the batch potency test.

2-3-1-3 Serological assay. Use not fewer than 35 fish from a population that does not have specific antibodies against *M. viscosa* and that are within specified limits for body mass. Carry out the test at a defined temperature. Inject intraperitoneally into each of not fewer than 25 fish a defined 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 after vaccination. Determine for each sample the level of specific antibodies against *M. viscosa* by a suitable immunochemical method (*2.7.1*). 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 for 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.