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Enteric Redmouth Disease Vaccine for Rainbow Trout (Inactivated)

General Notices

Enteric Redmouth Disease Vaccine (Inactivated) for Rainbow Trout (Ph. Eur. monograph 1950)

Ph Eur

1 DEFINITION

Enteric redmouth (ERM) disease vaccine (inactivated) for rainbow trout is prepared from cultures of serovars 1 or 2 of *Yersinia ruckeri*, inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for administration by injection or immersion for the active immunisation of rainbow trout against enteric redmouth disease.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The strains of *Y. ruckeri* are harvested and 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.

2-2 CHOICE OF VACCINE COMPOSITION

The strains of *Y. ruckeri* used 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).

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 rainbow trout with an average body mass corresponding to 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 under the conditions to be recommended for use of the vaccine with a water temperature not less than 10 °C.

2-2-1-1-1 Vaccines intended for administration by injection. Use not fewer than 50 fish from a population that does not have specific antibodies against the relevant serovars of *Y. ruckeri* and has not been vaccinated against or exposed to ERM disease. Where the size of the fish for the test is such that a blood sample cannot be removed for antibody testing, a

number of larger fish may be kept with the group for this purpose. 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 does not have specific antibodies against the relevant serovars of *Y. ruckeri* and has not been vaccinated against or exposed to ERM disease. Where the size of the fish for the test is such that a blood sample cannot be removed for antibody testing, a number of larger fish may be kept with the group for this purpose. 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.

2-2-2 Immunogenicity

Carry out a 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. Where the size of the fish for the test is such that a blood sample cannot be removed for antibody testing, a number of larger fish of the same origin may be selected for this purpose. 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 the relevant serovars of *Y. ruckeri* and has not been vaccinated against or exposed to ERM disease. The average body mass of the fish corresponds to the minimum body mass to be recommended for vaccination. 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 may 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 injection or immersion, with a sufficient quantity of cultures of *Y. ruckeri* of which the virulence has been verified or, where all fish are kept in the same tank, with a sufficient challenge by cohabitation. 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 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 1st death in the 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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For serovar 1 vaccines, the vaccine complies with the test if the RPS is not less than 75 per cent for vaccines administered by immersion and 90 per cent for vaccines administered by injection.

For serovar 2 vaccines, the vaccine complies with the test if the RPS is not less than 60 per cent for vaccines administered by immersion and 85 per cent for vaccines administered by injection.

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 the vaccine. Where the test is not carried out, an alternative validated method in fish or other vertebrate animals 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 25 fish from a population that does not have specific antibodies against the relevant serovars of *Y. ruckeri* and that are within specified limits for body mass. Carry out the test at a defined temperature. Inject into each of not fewer than 20 fish 1 dose of vaccine, according to the instructions for use. Perform mock vaccination on a control group of not fewer than 5 fish. Collect blood samples at a defined time after vaccination. Determine for each sample the level of specific antibodies against the relevant serovars of *Y. ruckeri* included in the vaccine by a suitable immunochemical method (*2.7.1*).

The test is not valid if the control group shows antibodies against the relevant serovars of *Y. ruckeri*. 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 has given 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 a 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.