For tetanus vaccine presented as a combined vaccine for use in animals other than horses, the above test may be carried out in susceptible rabbits instead of guinea-pigs. The vaccine complies with the test if the average antibody titre of the sera of the vaccinated rabbits is not less than 2.5 IU/ml.

01/2005:1580

**VIBRIOSES (COLD-WATER) VACCINE (INACTIVATED) FOR SALMONIDS**

**Vaccinum vibriosidis aquae frigidae inactivatum ad salmonidas**

**DEFINITION**

Cold-water vibriosis vaccine (inactivated) for salmonids is prepared from cultures of one or more suitable strains of *Vibrio salmonicida*.

**PRODUCTION**

The strains of *V. 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.

**CHOICE OF VACCINE COMPOSITION**

The strains or strains of *V. salmonicida* used are shown to be suitable with respect to production of antigens of assumed immunological importance. The vaccine is shown to be satisfactory with respect to safety (5.2.6) and immunogenicity (5.2.7) in the species of fish for which it is intended. The following tests may be used during demonstration of safety and immunogenicity:

- **Safety.** Safety is tested in three different batches using test A, test B or both, depending on the recommendations for use.
  - **A. Vaccines intended for administration by injection.**
    - A test is carried out in each species of fish for which the vaccine is intended. The fish used are from a population that does not have specific antibodies against *V. salmonicida* and which has not been vaccinated against nor exposed to cold-water vibriosis. The test is carried out in the conditions recommended for the use of the vaccine with a water temperature not less than 10 °C. An amount of vaccine corresponding to twice the recommended dose per mass unit for fish of the minimum body mass recommended for vaccination is administered intraperitoneally to each of not fewer than fifty fish of the minimum recommended body mass. The fish are observed for 21 days. No abnormal local or systemic reaction occurs. The test is invalid if more than 6 per cent of the fish die from causes not attributable to the vaccine.
  - **B. Vaccines intended for administration by immersion.**
    - A test is carried out in each species of fish for which the vaccine is intended. The fish used are from a population that does not have specific antibodies against *V. salmonicida* and which has not been vaccinated against nor exposed to cold-water vibriosis. The test is carried out in the conditions recommended for the use of the vaccine with a water temperature not less than 10 °C. Prepare an immersion bath at twice the recommended concentration. Not fewer than fifty fish, having not less than the minimum body mass recommended for vaccination are used. The fish are bathed for twice the recommended time. The fish are observed for 21 days. No abnormal local or systemic reaction occurs. The test is invalid if more than 6 per cent of the fish die from causes not attributable to the vaccine.

- **C. Safety is demonstrated in addition in field trials by administering the intended dose to a sufficient number of fish distributed in not fewer than two sets of premises. No abnormal reaction occurs.**

**Immunogenicity.** The test described under Potency, carried out for each recommended route of administration, is suitable to demonstrate immunogenicity of the vaccine.

**BATCH TESTING**

**Batch potency test.** For routine testing of batches of vaccine, the test described under Potency may be carried out using groups of not fewer than thirty fish of one of the species for which the vaccine is intended; alternatively, a suitable validated test based on antibody response may be carried out, the criteria 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 after a satisfactory correlation with the test described under Potency has been established.

Use fish from a population that does not have specific antibodies against *V. salmonicida* and that are within specified limits for body mass. Carry out the test at a defined temperature. Inject into each of not fewer than twenty-five fish one dose of vaccine, according to the instructions for use. Perform mock vaccination on a control group of not fewer than ten fish. Collect blood samples at a defined time after vaccination. Determine for each sample the level of specific antibodies against *V. salmonicida* by a suitable immunochemical method (2.7.1). The vaccine complies with the test if the mean level of antibodies is not significantly lower than that found for a batch that gave satisfactory results in the test described under Potency. The test is not valid if the control group shows antibodies against *V. salmonicida*.

**IDENTIFICATION**

When injected into fish that do not have specific antibodies against *V. salmonicida*, the vaccine stimulates the production of such antibodies.

**TESTS**

- **Safety.** Use not fewer than ten fish of one of the species for which the vaccine is intended, having, where possible, the minimum body mass recommended for vaccination; if fish of the minimum body mass are not available, use fish not greater than twice this mass. Use fish from a population that does not have specific antibodies against *V. salmonicida* and that has not been vaccinated against nor exposed to cold-water vibriosis. Carry out the test in the conditions recommended for use of the vaccine with a water temperature not less than 10 °C. For vaccines administered by injection or immersion, inject intraperitoneally into each fish an amount of vaccine corresponding to twice the recommended dose per mass unit. For vaccines administered by immersion only, use a bath with double the recommended concentration and bathe the fish for twice the recommended immersion time. Observe the animals for 21 days. No abnormal local or systemic reaction attributable to the vaccine occurs. The test is invalid if more than 10 per cent of the fish die from causes not attributable to the vaccine.

- **Sterility.** The vaccine complies with the test for sterility prescribed in the monograph on Vaccines for veterinary use (0062).

**POTENCY**

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. Vaccinate not fewer than one hundred fish by a
recommended route, according to the instructions for use. Perform mock vaccination on a control group of not fewer than one hundred 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. Carry out challenge by injection at a fixed time interval after vaccination, defined according to the statement regarding development of immunity. Use for challenge a culture of *V. salmonicida* whose virulence has been verified. Observe the fish 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 invalid if the specific mortality is less than 60 per cent in the control group 21 days after the first 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) from the expression:

\[
\left(1 - \frac{M}{60}\right) \times 106
\]

The vaccine complies with the test if the RPS is not less than 60 per cent for vaccines administered by injection and 90 per cent for vaccines administered by immersion.

**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.

01/2005:1581

**VIBRIOSIS VACCINE (INACTIVATED) FOR SALMONIDS**

**Vaccinum vibriosidis ad salmonidas inactivatum**

**DEFINITION**

Vibriosis vaccine (inactivated) for salmonids is prepared from cultures of one or more suitable strains or serovars of *Vibrio anguillarum*; the vaccine may also include *Vibrio ordalii*.

**PRODUCTION**

The strains of *V. anguillarum* and *V. ordalii* are cultured and harvested separately. The harvests are inactivated by a suitable method. Whole or disrupted cells may be used and the vaccine may contain extracellular products of the bacterium released into the growth medium.

**CHOICE OF VACCINE COMPOSITION**

The strains of *V. anguillarum* and *V. ordalii* used are shown to be suitable with respect to production of antigens of assumed immunological importance. The vaccine is shown to be satisfactory with respect to safety (5.2.6) and immunogenicity (5.2.7) in the species of fish for which it is intended. The following tests may be used during demonstration of safety and immunogenicity.

**Safety.** Safety is tested in three different batches using test A, test B or both, depending on the recommendations for use.

A. Vaccines intended for administration by injection. A test is carried out in each species of fish for which the vaccine is intended. The fish used are from a population that does not have specific antibodies against the relevant serovars of *V. anguillarum* or where applicable *V. ordalii* and which has not been vaccinated against nor exposed to vibriosis. The test is carried out in the conditions recommended for the use of the vaccine with a water temperature not less than 10 °C. An amount of vaccine corresponding to twice the recommended dose per mass unit for fish of the minimum body mass recommended for vaccination is administered intraperitoneally to each of not fewer than fifty fish of the minimum recommended body mass. The fish are observed for 21 days. No abnormal local or systemic reaction occurs. The test is invalid if more than 6 per cent of the fish die from causes not attributable to the vaccine.

B. Vaccines intended for administration by immersion. A test is carried out in each species of fish for which the vaccine is intended. The fish used are from a population that does not have specific antibodies against the relevant serovars of *V. anguillarum* or where applicable *V. ordalii* and which has not been vaccinated against nor exposed to vibriosis. The test is carried out in the conditions recommended for the use of the vaccine with a water temperature not less than 10 °C. Prepare an immersion bath at twice the recommended concentration. Not fewer than fifty fish having the minimum body mass recommended for vaccination are used. The fish are bathed for twice the recommended time. The fish are observed for 21 days. No abnormal local or systemic reaction occurs. The test is invalid if more than 6 per cent of the fish die from causes not attributable to the vaccine.

C. Safety is also demonstrated in field trials by administering the intended dose to a sufficient number of fish distributed in not fewer than two sets of premises. No abnormal reaction occurs.

**Immunogenicity.** The test described under Potency, carried out for each recommended route of administration is suitable to demonstrate immunogenicity of the vaccine.

**BATCH TESTING**

**Batch potency test.** For routine testing of batches of vaccine, the test described under Potency may be carried out using groups of not fewer than thirty fish of one of the species for which the vaccine is intended; alternatively, a suitable validated test based on antibody response may be carried out. The criteria 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 after a satisfactory correlation with the test described under Potency has been established.

Use fish from a population that does not have specific antibodies against the relevant serovars of *V. anguillarum* and where applicable *V. ordalii* and that are within specified limits for body mass. Carry out the test at a defined temperature. Inject into each of not fewer than twenty-five fish one dose of vaccine, according to the instructions for use. Perform mock vaccination on a control group of not fewer than ten fish. Collect blood samples at a defined time after vaccination. Determine for each sample the level of specific antibodies against the different serovars of *V. anguillarum* and against *V. ordalii* included in the vaccine by a suitable immunochemical method (2.7.1). The vaccine complies with the test if the mean levels of antibodies are not significantly lower than those found for a batch that gave satisfactory results in the test described under Potency. The test is not valid if the control group shows antibodies against the relevant serovars of *V. anguillarum* or, where applicable, against *V. ordalii*. 

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**NOTE:** General Notices (1) apply to all monographs and other texts 797

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**Vibrio ordalii**