The vaccine contains an oily adjuvant.

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 strains of A. salmonicida subsp. salmonicida may be used and the vaccine may contain extracellular products of the bacterium released into the growth medium. 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 may be used during demonstration of safety and immunogenicity.

Safety
A. During development of the vaccine, safety is tested on 3 different batches. 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 A. salmonicida subsp. salmonicida and has not been vaccinated against or exposed to furunculosis. 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 is administered intraperitoneally into each of not fewer than 50 fish of the minimum body mass recommended for vaccination. 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. 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.

Immunogenicity
The test described under Potency is suitable to demonstrate the 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 not fewer than 30 fish per group; 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 A. salmonicida subsp. salmonicida and that are within defined limits for body mass. Carry out the test at a defined temperature. Inject intraperitoneally into each of not fewer than 25 fish one 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 at a defined time 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 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 A. salmonicida subsp. salmonicida.
IDENTIFICATION

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

TESTS

Safety. Use not fewer than 10 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 preferably does not have specific antibodies against *A. salmonicida* subsp. *salmonicida* or, where justified, use fish from a population with a low level of such antibodies as long as they have not been vaccinated against or exposed to furunculosis and administration of the vaccine does not cause an anamnestic response. Carry out the test in the conditions recommended for use of the vaccine with a water temperature not less than 10 °C. Administer intraperitoneally to each fish an amount of vaccine corresponding to twice the recommended dose per mass unit. 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 100 fish by a recommended route, according to the instructions for use. Perform mock vaccination on a control group of not fewer than 100 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 *A. salmonicida* subsp. *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:

\[ \left(1 - \frac{M}{60}\right) \times 100 \]

The vaccine complies with the test if the RPS is not less than 80 per cent.

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.

INFECTIOUS BOVINE RHINOTRACHEITIS VACCINE (LIVE), FREEZE-DRIED

*Vaccinum rhinotracheitidis infectivea bovinae vivum cryodesiccatum*

**DEFINITION**

Freeze-dried infectious bovine rhinotracheitis vaccine (live) is a preparation of one or more attenuated strains of infectious bovine rhinotracheitis virus (bovine herpesvirus 1).

**PRODUCTION**

The virus is grown in suitable cell cultures (5.2.4). The viral suspension is collected and mixed with a suitable stabilising solution. The mixture is subsequently freeze-dried.

**CHOICE OF VACCINE STRAIN**

Only a virus strain shown to be satisfactory with respect to the following characteristics may be used in the preparation of the vaccine: safety (including absence of abortigenicity and passage through the placenta); reversion to virulence and immunogenicity. The strain may have markers. The following tests may be used during demonstration of safety (5.2.6) and efficacy (5.2.7).

Safety. Use 5 calves 3 months old, or of the minimum age to be recommended for vaccination if this is less than 3 months, and that do not have antibodies against infectious bovine rhinotracheitis virus. Administer to each calf, by the intended route, a quantity of virus corresponding to 10 doses of vaccine. The calves are observed for 21 days. No abnormal local or systemic reaction occurs.

Abortigenicity and passage through the placenta. 24 pregnant cows that do not have antibodies against infectious bovine rhinotracheitis virus are used for the test: 8 of the cows are in the fourth month of pregnancy, 8 in the fifth month and 8 in the sixth or seventh month. A quantity of virus equivalent to ten doses of vaccine is administered by the intended route to each cow. The cows are observed until the end of pregnancy. If abortion occurs, tests for infectious bovine rhinotracheitis virus are carried out; neither the virus nor viral antigens are present in the foetus or placenta. A test for antibodies against infectious bovine rhinotracheitis virus is carried out on calves born at term before ingestion of colostrum; no such antibodies are found.

Reversion to virulence. Suitable samples are taken from the 5 calves used for the test for safety at a time when the vaccinal virus can be easily detected. The presence and titre of virus in the samples are verified. The samples are then mixed and administered intranasally to 2 other calves of the same age having no antibodies against bovine rhinotracheitis virus. 5 further serial passages are carried out. The presence of the virus is verified at each passage. No abnormal local or systemic reaction occurs.

Immunogenicity. The test described under Potency is suitable to demonstrate immunogenicity.

**BATCH TESTING**

If the test for potency has been carried out with satisfactory results on a representative batch of vaccine, this test may be omitted as a routine control on other batches of vaccine prepared from the same seed lot, subject to agreement by the competent authority.