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.
IDENTIFICATION
A. Reconstitute the vaccine as stated on the label. When mixed with a suitable quantity of a monospecific antiserum, the reconstituted vaccine is no longer able to infect susceptible cell cultures into which it is inoculated.

B. Any markers of the strain are verified.

TESTS
Safety. Use 2 calves 3 months old or of the minimum age recommended for vaccination if this is less than 3 months, having no antibodies against bovine rhinotracheitis virus. Administer 10 doses of the reconstituted vaccine to each calf by a recommended route. Observe the calves for 21 days. No abnormal local or systemic reaction occurs.

Bacterial and fungal contamination. The reconstituted vaccine complies with the test for sterility prescribed in the monograph on Vaccines for veterinary use (0062).

Mycoplasmas (2.6.7). The reconstituted vaccine complies with the test for mycoplasmas.

Extraneous viruses. Neutralise the vaccine using a monospecific antiserum and inoculate into suitable cell cultures. Maintain the cultures for 14 days and make a passage at 7 days. The cell cultures show no signs of viral contamination.

Virus titre. Titrate the reconstituted vaccine in susceptible cell cultures at a temperature favourable to replication of the virus. One dose of the vaccine contains not less than the quantity of virus equivalent to the minimum virus titre stated on the label.

POTENCY
Use susceptible calves, 2 to 3 months old and free from antibodies neutralising infectious bovine rhinotracheitis virus. Administer to five calves, by the route stated on the label a volume of the reconstituted vaccine containing a quantity of virus equivalent to the minimum virus titre stated on the label. Keep 2 calves as controls. After 21 days, administer intranasally to the seven calves a quantity of infectious bovine rhinotracheitis virus sufficient to produce typical signs of disease such as fever, ocular and nasal discharge and ulceration of the nasal mucosa in a susceptible calf. Observe the animals for 21 days. The vaccinated calves show no more than mild signs; the controls show typical signs. In not fewer than 4 of the 5 vaccinated calves, the maximum virus titre found in the nasal mucus is at least 10 times lower than the average of the maximum titres found in the control calves; the average number of days on which virus is excreted is at least 3 days less in vaccinated calves than in the control calves.

INFEKTIOUS CHICKEN ANAEMIA VACCINE (LIVE)

Vaccinum anaemiae infectivae pulli vivum

1. DEFINITION
Infectious chicken anaemia vaccine (live) is a preparation of a suitable strain of chicken anaemia virus. This monograph applies to vaccines intended for administration to breeder chickens for active immunisation, to prevent excretion of the virus, to prevent or reduce egg transmission and to protect passively their future progeny.

2. PRODUCTION
2.1. PREPARATION OF THE VACCINE
The vaccine virus is grown in embryonated hens' eggs or in cell cultures.

2.2. SUBSTRATE FOR VIRUS PROPAGATION
2.2.1. Embryonated hens' eggs. If the vaccine virus is grown in embryonated hens' eggs, they are obtained from flocks free from specified pathogens (SPF) (5.2.2).

2.2.2. Cell cultures. If the vaccine virus is grown in cell cultures, they comply with the requirements for cell cultures for production of veterinary vaccines (5.2.4).

2.3. SEED LOTS
2.3.1. Extraneous agents. The master seed lot complies with the tests for extraneous agents in seed lots (2.6.24). In these tests on the master seed lot, the organisms used are not more than 5 passages from the master seed lot at the start of the tests.

2.4. CHOICE OF VACCINE VIRUS
The vaccine virus shall be shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for the chickens for which it is intended.

The following tests for safety (section 2-4.1), increase in virulence (section 2-4.2) and immunogenicity (section 2-4.3) may be used during the demonstration of safety and immunogenicity.

2.4.1. Safety
2.4.1. General test. Carry out the test for each route and method of administration to be recommended for vaccination in chickens not older than the youngest age to be recommended for vaccination and from an SPF flock (5.2.2). Use vaccine virus at the least attenuated passage level that will be present between the master seed lot and a batch of the vaccine. For each test use not fewer than 20 chickens. Administer to each chicken a quantity of the vaccine virus not less than 10 times the maximum virus titre likely to be contained in 1 dose of the vaccine. 14 days after vaccination, collect blood samples from half of the chickens and determine the haematocrit value. Kill these chickens and carry out post-mortem examination. Note any pathological changes attributable to chicken anaemia virus, such as thymic atrophy and specific bone-marrow lesions. Observe the remaining chickens at least daily for 21 days. The vaccine virus complies with the test if during the observation period no chicken shows notable clinical signs of chicken anaemia or dies from causes attributable to the vaccine virus.

2.4.1.2. Safety for young chicks. Use not fewer than twenty 1-day-old chicks from an SPF flock (5.2.2). Administer to each chick by the oculonasal route a quantity of the vaccine virus equivalent to not less than the maximum titre likely to be contained in 1 dose of the vaccine. 14 days after vaccination, collect blood samples from half of the chicks and determine the haematocrit value. Kill these chicks and carry out post-mortem examination. Note any pathological changes attributable to chicken anaemia virus, such as thymic atrophy and specific bone-marrow lesions. Observe the remaining chicks at least daily for 21 days. The vaccine virus complies with the test if during the observation period no chicken shows notable clinical signs of chicken anaemia or dies from causes attributable to the vaccine virus.

2.4.2. Immunogenicity.
2.4.2. Choice of vaccine virus. For the purposes of this monograph, the vaccine virus is chosen on the basis of immunogenicity. Immunogenicity is assessed in chickens by determining the proportion of chicks examined at 14 days that show typical signs of chicken anaemia, such as depression, decreased appetite, diarrhea, and haemorrhages in the lungs. Observe the remaining chicks at least daily for 21 days. The vaccine virus complies with the test if during the observation period no chicken shows notable clinical signs of chicken anaemia or dies from causes attributable to the vaccine virus.

2.4.2.2. Resistance of the vaccine virus to extraneous agents. The vaccine virus is resistant to extraneous agents in the following tests:

(a) In embryonated hens' eggs, they are obtained from flocks free from specified pathogens (SPF) (5.2.2). In these tests on the master seed lot, the organisms used are not more than 5 passages from the master seed lot at the start of the tests.

(b) In cell cultures, they comply with the requirements for cell cultures for production of veterinary vaccines (5.2.4).