If the virus titre is determined in plaque-forming units (PFU), vaccine virus by inoculation into suitable cell cultures (5.2.2). Vaccinate by a recommended route not fewer than 30 chickens. Maintain not fewer than 30 chickens as controls. Challenge each chicken after 9 days by a suitable route with a sufficient quantity of virulent Marek's disease virus. Observe the chickens at least daily for 70 days after challenge. Record the deaths and the number of surviving chickens that show clinical signs of disease. At the end of the observation period kill all the surviving chickens and carry out examination for macroscopic lesions of Marek's disease. The test is not valid if:

- during the observation period after challenge fewer than 70 per cent of the control chickens die or show severe clinical signs or macroscopic lesions of Marek's disease,
- and/or during the period between the vaccination and challenge more than 10 per cent of the control or vaccinated chickens show abnormal clinical signs or die from causes not attributable to the vaccine.

The vaccine virus complies with the test if the percentage relative protection, calculated using the following expression, is not less than 80 per cent:

\[
V = \frac{V - C}{100 - C} \times 100
\]

\[V\] = percentage of challenged vaccinated chickens that survive to the end of the observation period without notable clinical signs or macroscopic lesions of Marek's disease,

\[C\] = percentage of challenged control chickens that survive to the end of the observation period without notable clinical signs or macroscopic lesions of Marek's disease.

3. BATCH TESTS

3-1. Identification. Carry out an immunostaining test in cell cultures using monoclonal antibodies to demonstrate the presence of each type of virus stated on the label.

3-2. Bacteria and fungi. The vaccine and, where applicable, the liquid supplied with it comply with the requirement for sterility prescribed in the monograph Vaccines for veterinary use (0062).

3-3. Mycoplasmas. The vaccine complies with the test for mycoplasmas (2.6.7).

3-4. Extraneous agents. The vaccine complies with the tests for extraneous agents in batches of finished product (2.6.25).

3-5. Safety. Use not fewer than 10 chickens from an SPF flock (5.2.2) and not older than the youngest age recommended for vaccination. Administer by a recommended route and method to each chicken 10 doses of the vaccine. Observe the chickens at least daily for 21 days. The test is not valid if more than 20 per cent of the chickens show abnormal clinical signs or die from causes not attributable to the vaccine. The vaccine complies with the test if no chicken shows notable clinical signs of disease or dies from causes attributable to the vaccine.

3-6. Virus titre

3-6-1 Vaccines containing one type of virus. Titrate the vaccine virus by inoculation into suitable cell cultures (5.2.4). If the virus titre is determined in plaque-forming units (PFU), only primary plaques are taken into consideration. The vaccine complies with the test if 1 dose contains not less than the minimum virus titre stated on the label.

3-6-2. Vaccines containing more than one type of virus. For vaccines containing more than 1 type of virus, titrate each virus by inoculation into suitable cell cultures (5.2.4), reading the results by immunostaining using antibodies. The vaccine complies with the test if 1 dose contains for each vaccine virus not less than the minimum virus titre stated on the label.

3-7. Potency. The vaccine complies with the requirements of the test prescribed under Immunogenicity (section 2.4-3) when administered according to the recommended schedule by a recommended route and method. It is not necessary to carry out the potency test for each batch of the vaccine if it has been carried out on a representative batch using a vaccinating dose containing not more than the minimum virus titre stated on the label.

**MYXOMATOSIS VACCINE (LIVE) FOR RABBITS**

**Vaccinum myxomatosisidis vivum ad cuniculum**

**DEFINITION**

Myxomatosis vaccine (live) for rabbits is a preparation of a suitable strain of either myxoma virus that is attenuated for rabbits or Shope fibroma virus. The vaccine is intended for the active immunisation of rabbits against myxomatosis.

**PRODUCTION**

The virus is propagated in suitable cell cultures (5.2.4). The viral suspension is harvested, titrated and may be mixed with a suitable stabilising solution. The vaccine may be freeze-dried.

**CHOICE OF VACCINE STRAIN**

The vaccine is shown to be satisfactory with respect to safety, absence of increase in virulence and immunogenicity. The following tests may be used during demonstration of safety, absence of increase in virulence (5.2.6) and efficacy (5.2.7). Safety. The test is carried out for each route of administration to be stated on the label. Use at least 10 rabbits of the minimum age to be recommended for vaccination and that do not have antibodies against myxoma virus. Administer to each rabbit by a recommended route a quantity of virus corresponding to not less than 10 times the maximum titre that may be expected in a dose of vaccine. Observe the rabbits for 28 days. Record the body temperature the day before vaccination, at vaccination, 4 h after vaccination and then daily for 4 days; note the maximum temperature increase for each animal. No abnormal local or systemic reaction occurs; the average temperature increase does not exceed 1 °C and no animal shows a rise greater than 2 °C. A local reaction lasting less than 28 days may occur.

If the vaccine is intended for use in pregnant rabbits, administer the virus to not less than 10 pregnant rabbits according to the schedule to be recommended on the label. Prolong the observation period until 1 day after parturition. The rabbits remain in good health and there is no abnormal local or systemic reaction. No adverse effects on the pregnancy or the offspring are noted.

Increase in virulence. (This test is performed only for vaccines based on attenuated strains of myxoma virus). Administer by a recommended route to each of 2 rabbits, 5 to 7 weeks old and which do not have antibodies against...
myxoma virus, a quantity of virus that will allow recovery of virus for the passages described below. Use vaccine virus at the least attenuated passage level that will be present between the master seed lot and a batch of the vaccine. Kill the rabbits 5 to 10 days after inoculation and remove from each rabbit organs, or tissues with sufficient virus to allow passage: homogenise the organs and tissues in a suitable buffer solution, centrifuge the suspension and use the supernatant for further passages. Inoculate the supernatant into suitable cell cultures to verify the presence of virus. Administer by an appropriate route, at a suitable rate, a suitable volume of the supernatant to each of 2 other rabbits of the same age and the same susceptibility. This operation is then repeated at least 5 times. If the virus has disappeared, a second series of passages is carried out. Inoculate virus from the highest recovered passage level to rabbits, observe for 28 days and compare any reactions that occur with those seen in the test for safety described above. There is no indication of an increase in virulence as compared with the non-passaged virus. If virus is not recovered in either of 2 series of passages, the vaccine virus also complies with the test.

**Immunogenicity.** The test described under Potency may be used to demonstrate the immunogenicity of the strain.

**BATCH POTENCY TEST**

If the test for potency has been carried out with satisfactory results on a representative batch of vaccine, using a vaccinating dose containing not more than the minimum virus titre stated on the label, this test may be omitted as a routine control on other batches of vaccine prepared from the same seed lot.

**IDENTIFICATION**

Carry out an immunofluorescence test in suitable cell cultures, using a monospecific antiserum.

**TESTS**

**Safety.** Use not fewer than 2 rabbits, not older than the minimum age recommended for vaccination, that do not have antibodies against myxoma virus and rabbit haemorrhagic disease virus and that have been reared in suitable isolation conditions to avoid contact with myxoma virus. Administer by a recommended route to each rabbit 10 doses of vaccine. Observe the rabbits at least daily for 14 days. No abnormal local or systemic reaction occurs.

**Extraneous agents.** At the end of the 14 day observation period of the safety test, administer by a recommended route to each rabbit a further 10 doses of vaccine. After 14 days take a blood sample from each rabbit and carry out a test for antibodies against rabbit haemorrhagic disease virus. No antibodies are found.

**Bacterial and fungal contamination.** The vaccine, reconstituted if necessary, complies with the test for sterility prescribed in the monograph on Vaccines for Veterinary Use (0062).

**Mycoplasmas (2.6.7).** The vaccine, reconstituted if necessary, complies with the test for mycoplasmas.

**Virus titre.** Reconstitute the vaccine, if necessary, and titrate in suitable cell cultures. 1 dose of the vaccine contains not less than the quantity of virus equivalent to the minimum virus titre stated on the label.

**POTENCY**

Use not fewer than 15 susceptible rabbits of the minimum age to be recommended for vaccination, free from antibodies against myxoma virus and reared in suitable isolation conditions to ensure absence of contact with myxoma virus.Administer 1 dose of vaccine to each of not fewer than 10 of the rabbits according to the instructions for use. Keep not less than 5 other rabbits as controls. Not less than 21 days after the last vaccination, administer by a suitable route to each rabbit a quantity of a virulent strain of myxoma virus sufficient to cause typical signs of myxomatosis in a susceptible rabbit. Observe the rabbits for a further 21 days. The test is not valid if fewer than 90 per cent of the control rabbits display typical signs of myxomatosis. A vaccine containing myxoma virus complies with the test if not fewer than 90 per cent of vaccinated rabbits show no signs of myxomatosis. A vaccine containing Shope fibroma virus complies with the test if not fewer than 75 per cent of vaccinated rabbits show no signs of myxomatosis.

**LABELLING**

The label states, where applicable, that a local reaction may occur.

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**NEONATAL PIGLET COLIBACILLOSIS VACCINE (INACTIVATED)**

Vaccinum colibacillosis fetus a partu recentis inactivatum ad suem

**DEFINITION**

Neonatal piglet colibacillosis vaccine (inactivated) is prepared from cultures of one or more suitable strains of Escherichia coli, carrying one or more adhesins or enterotoxins. This monograph applies to vaccines administered by injection to sows and gilts for protection of newborn piglets against enteric forms of colibacillosis.

**PRODUCTION**

The E. coli strains used for production are cultured separately in a suitable medium. The cells or toxins are processed to render them safe and are blended.

The vaccine may contain one or more suitable adjuvants.

**CHOICE OF VACCINE COMPOSITION**

The E. coli strains used in the production of the vaccine are shown to be satisfactory with respect to expression of antigens and the vaccine is shown to be satisfactory with respect to safety and immunogenicity. The following tests may be used during demonstration of safety (5.2.6) and efficacy (5.2.7).

**Expression of antigens.** The expression of antigens that stimulate a protective immune response is verified by a suitable immunochemical method (2.7.1) carried out on the antigen obtained from each of the vaccine strains under the conditions used for the production of the vaccine.

**Safety**

A. Administer a double dose of vaccine by a recommended route to each of not fewer than 10 pregnant sows that have not been vaccinated against colibacillosis. Administer 1 dose of vaccine to each of the animals after the recommended interval. Observe the animals until farrowing. Record body temperature the day before vaccination, at vaccination, 2 h, 4 h and 6 h later and then daily for 4 days; note the maximum temperature increase for each animal. Note any effects on gestation or the offspring. No abnormal local or systemic reaction occurs: the average temperature increase for all animals does not exceed 1.5 °C and no animal shows a rise greater than 2 °C.