Edition: BP 2025 (Ph. Eur. 11.6 update)

Canine Parvovirus Vaccine, Inactivated

General Notices

(Canine Parvovirosis Vaccine (Inactivated), Ph. Eur. monograph 0795)

Ph Eur

1 DEFINITION

Canine parvovirosis vaccine (inactivated) is a preparation of a suitable strain of canine parvovirus inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of dogs against canine parvovirosis.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The vaccine virus is grown in cell cultures. The virus harvest is inactivated. The vaccine may be adjuvanted.

2-2 SUBSTRATE FOR VIRUS PROPAGATION

2-2-1 Cell cultures

The cell cultures comply with the requirements for cell cultures for production of veterinary vaccines (*5.2.4*).

2-3 CHOICE OF VACCINE COMPOSITION

The vaccine is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*) for the dogs for which it is intended.

The following tests for safety (section 2-3-1) and immunogenicity (section 2-3-2) may be used during the demonstration of safety and efficacy.

2-3-1 Safety

Carry out the test for each route and method of administration 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.

For each test, use not fewer than 8 dogs not older than the minimum age to be recommended for vaccination and that do not have antibodies against canine parvovirus. Administer to each dog 1 dose of the vaccine. If the schedule to be recommended requires a 2nd dose, administer 1 dose after an interval of at least 14 days. Observe the dogs at least daily for at least 14 days after the last administration.

The vaccine complies with the test if no dog shows abnormal local or systemic reactions or dies from causes attributable to the vaccine.

2-3-2 Immunogenicity

A test is carried out for each route and method of administration to be recommended for vaccination using in each case dogs of the minimum age to be recommended. The vaccine administered to each dog is of minimum potency.

Use for the test not fewer than 7 dogs that do not have antibodies against canine parvovirus. Vaccinate not fewer than 5 dogs according to the schedule to be recommended. Maintain not fewer than 2 dogs as controls. Challenge each dog after 20-22 days by the oronasal route with a sufficient quantity of a suspension of pathogenic canine parvovirus. Observe the dogs at least daily for 14 days after challenge. At the end of the observation period, carry out haemagglutination tests for and titration of the virus in the faeces.

The test is not valid if fewer than 100 per cent of the control dogs show notable signs of the disease or leucopenia and excretion of the virus. The vaccine complies with the test if all the vaccinated dogs survive and show no signs of disease nor leucopenia and if the maximum titre of virus excreted in the faeces is less than 1/100 of the geometric mean of the maximum titres found in the controls.

2-4 MANUFACTURER'S TESTS

2-4-1 Residual live virus

A test for residual live virus is carried out on the bulk harvest of each batch. The quantity of inactivated virus harvest used in the test is equivalent to not less than 100 doses of the vaccine. The inactivated virus harvest is inoculated into suitable non-confluent cells; after incubation for 8 days, a subculture is made using trypsinised cells. After incubation for a further 8 days, the cultures are examined for residual live parvovirus by an immunofluorescence test. The immunofluorescence test may be supplemented by a haemagglutination test or other suitable tests on the supernatant of the cell cultures. The inactivated virus harvest complies with the test if no live virus is detected.

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 Residual live virus

This test may be omitted for batch release, as stated in the monograph Vaccines for veterinary use (0062).

Carry out a test for residual live virus by inoculating not less than 10 doses of vaccine into suitable non-confluent cells; after incubation for 8 days, a subculture is made using trypsinised cells. After incubation for a further 8 days, the cultures are examined for residual live parvovirus by an immunofluorescence test. The immunofluorescence test may be supplemented by a haemagglutination test or other suitable tests on the supernatant of the cell cultures. The vaccine complies with the test if no live virus is detected. If the vaccine contains an adjuvant that interferes with the test, separate it if possible from the liquid phase of the vaccine by a method that does not inactivate the virus or otherwise interfere with the detection of live viruses.

3-4 Potency

Carry out test 3-4-1 or test 3-4-2.

3-4-1 Test in guinea-pigs for haemagglutination-inhibiting antibodies. Use for the test not fewer than 5 guinea-pigs that do not have specific antibodies. Administer to each guinea-pig by the subcutaneous route half of the dose to be

recommended. After 14 days, inject again half of the dose to be recommended. 14 days later, collect blood samples and separate the serum. Inactivate each serum by heating at 56 °C for 30 min. To 1 volume of each serum add 9 volumes of a 200 g/L suspension of *light kaolin R* in *phosphate buffered saline pH 7.4 R*. Shake each mixture for 20 min. Centrifuge, collect the supernatant and mix with 1 volume of a concentrated suspension of pig erythrocytes. Allow to stand at 4 °C for 60 min and centrifuge. The dilution of the serum obtained is 1:10. Using each serum, prepare a series of twofold dilutions. To 0.025 mL of each of the latter dilutions add 0.025 mL of a suspension of canine parvovirus antigen containing 4 haemagglutinating units. Allow to stand at 37 °C for 30 min and add 0.05 mL of a suspension of pig erythrocytes containing 30×10^6 cells per millilitre. Allow to stand at 4 °C for 90 min and note the last dilution of serum that still completely inhibits haemagglutination. The vaccine complies with the test if the median antibody titre of the sera collected after the second vaccination is not less than 1/80.

3-4-2 Test in dogs for virus-neutralising antibodies. Use for the test not fewer than 2 healthy dogs, 8-12 weeks old, that have antibody titres less than 4 ND_{50} per 0.1 mL of serum, measured by the method described below. Vaccinate each dog according to the recommended schedule. 14 days after vaccination, examine the serum of each dog as follows. Heat the serum at 56 °C for 30 min and prepare serial dilutions using a medium suitable for canine cells. Add to each dilution an equal volume of a virus suspension containing an amount of virus such that when the volume of serum-virus mixture appropriate for the assay system is inoculated into cell cultures, each culture receives approximately 10^4 $CCID_{50}$. Incubate the mixtures at 37 °C for 1 h and inoculate 4 canine cell cultures with a suitable volume of each mixture. Incubate the cell cultures at 37 °C for 7 days, passage and incubate for a further 7 days. Examine the cultures for evidence of specific cytopathic effects and calculate the antibody titre. The vaccine complies with the test if the mean titre is not less than 32 ND_{50} per 0.1 mL of serum. If one dog fails to respond, repeat the test using 2 more dogs and calculate the result as the mean of the titres obtained from all of the 3 dogs that have responded.
