each puppy by a recommended route. Observe for 14 days. The puppies remain in good health and no abnormal local or systemic reaction occurs.

**Extraneous viruses.** Neutralise the vaccine virus using a monospecific antiserum and inoculate into cell cultures known for their susceptibility to viruses pathogenic for the dog. Carry out a passage after 6 to 8 days and maintain the cultures for a total of 14 days. No cytopathic effect develops and the cells show no evidence of the presence of haemadsorbing agents.

**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 1,000,000 virus equivalent to the minimum virus titre stated on the label.

**POTENCY**

Use not fewer than 15 susceptible puppies of the minimum age recommended for vaccination and which do not have antibodies against parainfluenza virus of canine origin. 

Vaccinate not fewer than 10 of the puppies according to the instructions for use. Keep not fewer than 5 other puppies as controls. Observe all the animals for not less than 21 days after the last vaccination. Administer by the intratracheal or intranasal route to each animal a quantity of a virulent strain of parainfluenza virus of canine origin sufficient to establish infection with the virus in a susceptible dog. Observe the animals for a further 14 days. Collect nasal swabs or washings from each dog daily from day 2 to 10 after challenge and test these samples for the presence of excreted virus. Use a scoring system to record the incidence of coughing in each dog. The test is not valid if more than 1 of the control animals shows neither coughing nor the excretion of the challenge virus. The vaccine complies with the test if the scores for coughing or virus excretion for the vaccinated animals are significantly lower than in the controls.

**Virus titre**

The virus is propagated in suitable cell cultures (5.2.4). The virus may be purified and concentrated.

A test for residual live virus is carried out on the bulk harvest of each batch to confirm inactivation of the canine parvovirus. The quantity of inactivated virus used in the test is equivalent to not less than 100 doses of the vaccine. The vaccine 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. No live virus is detected.

The vaccine may contain an adjuvant or adjuvants.

**CHOICE OF VACCINE COMPOSITION**

The vaccine is shown to be satisfactory with respect to safety and immunogenicity in dogs. The following test may be used in the demonstration of efficacy (5.2.7).

**Immunogenicity.** 7 susceptible dogs of the minimum age recommended for vaccination are used. A blood sample is drawn from each dog and tested individually for antibodies against canine parvovirus to determine susceptibility. 5 dogs are vaccinated according to the recommended schedule. 2 dogs are kept as controls. 20 to 22 days after the last vaccination each of the dogs receives by the oronasal route a suspension of pathogenic canine parvovirus. The dogs are observed for 14 days. Haemagglutination tests are carried out to detect virus in the faeces. The test is not valid unless the 2 control dogs show typical signs of the disease or leucopenia and excretion of the virus. The vaccine complies with the test if the 5 vaccinated dogs remain in excellent health and show no sign of the 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.

**IDENTIFICATION**

When injected into dogs, the vaccine stimulates the production of antibodies against canine parvovirus.

**TESTS**

**Safety.** Use dogs of the minimum age recommended for vaccination and preferably having no canine parvovirus antibodies or, where justified, use dogs with a low level of such antibodies as long as they have not been vaccinated against canine parvovirus and administration of the vaccine does not cause an anamnestic response. Administer a double dose of vaccine by a recommended route to each of 2 dogs. Observe the animals for 14 days. No abnormal local or systemic reaction occurs.

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

**POTENCY**

**CANINE PARVOVIROSIS VACCINE**

**(INACTIVATED)**

Vaccinum parvovirosis caninae inactivatum

**DEFINITION**

Inactivated canine parvovirosis vaccine is a liquid or freeze-dried preparation of canine parvovirus inactivated by a suitable method.

**PRODUCTION**

The virus is propagated in suitable cell cultures (5.2.4). The virus may be purified and concentrated.

A test for residual live virus is carried out on the bulk harvest of each batch to confirm inactivation of the canine parvovirus. The quantity of inactivated virus used in the test is equivalent to not less than 100 doses of the vaccine. The vaccine 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
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.

B. Vaccinate, according to the schedule stated on the label, 2 healthy susceptible dogs, 8 to 12 weeks old and having antibody titres less than 4 ND<sub>50</sub> (50 per cent neutralising dose) per 0.1 ml of serum measured by the method described below. 14 days after vaccination, examine the serum of each animal 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<sup>6</sup> CCID<sub>50</sub>. 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<sub>50</sub> 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.

01/2005:0964

CANINE PARVOVIRUS VACCINE (LIVE)

Vaccinum parvoviruses caninae vivum

DEFINITION

Canine parvovirus vaccine (live) is a preparation of a strain of canine parvovirus that is attenuated for the dog.

PRODUCTION

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

CHOICE OF VACCINE STRAIN

Only a virus strain shown to be satisfactory with respect to safety, irreversibility of attenuation, and immunogenicity may be used in the preparation of the vaccine. The following tests are used in the demonstration of safety (5.2.6) and efficacy (5.2.7).

Safety. Each test is carried out for each recommended route of administration.

5 susceptible puppies of the minimum age recommended for vaccination and having no haemagglutination-inhibiting antibodies against canine parvovirus are used for the test. A count of white blood cells in circulating blood is made on days 4, 2 and 0 before injection of the vaccine strain. Each puppy receives by a recommended route a quantity of virus corresponding to not less than 10 times the maximum virus titre that may be expected in a batch of vaccine and at the lowest passage level. The puppies are observed for 21 days. A count of white blood cells in circulating blood is made on days 3, 5, 7 and 10 after the injection. The puppies remain in good health and there is no abnormal local or systemic reaction. Any diminution in the number of circulating white blood cells is not greater than 50 per cent of the initial number determined as the average of the 3 values found before injection of the vaccine strain.

A quantity of virus corresponding to not less than 10 times the maximum titre that may be expected in a batch of vaccine and at the lowest passage level is administered by one of the recommended routes to each of 5 susceptible puppies. Five puppies are kept as controls. 2 puppies from each group are killed at 14 days and the 3 remaining puppies from each group at 21 days and histological examination of the thymus of each animal is carried out. Slight hypoplasia of the thymus may be evident after 14 days. The strain is not acceptable if damage is evident after 21 days.

Irreversibility of attenuation. Use 2 susceptible puppies of the minimum age recommended for vaccination and which do not have haemagglutination-inhibiting antibodies against canine parvovirus. Administer to each puppy, by a recommended route, a quantity of virus corresponding to 10 times the maximum titre that may be expected in a batch of vaccine. From the second to the tenth day after administration of the virus, the faeces are collected from each puppy and checked for the presence of the virus; faeces containing virus are pooled. 1 ml of the suspension of pooled faeces is administered by the oronasal route to each of 2 other puppies of the same age and susceptibility; this operation is carried out 4 times. The presence of virus is verified at each passage. If the virus is not found, a second identification of passages is carried out; if the virus is not found in one of the second identification of passages, the vaccine strain complies with the test. No puppy dies or shows signs attributable to the vaccine. No indication of increase of virulence compared to the original vaccinal virus is observed; account is taken, notably, of the count of white blood cells, of results of histological examination of the thymus and of the titre of excreted virus.

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

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.

IDENTIFICATION

The vaccine is grown in a susceptible cell line in a substrate suitable for presenting for fluorescent antibody or immunoperoxidase tests. Suitable controls are included. A proportion of the cells is tested with a monoclonal antibody specific for canine parvovirus and a proportion of the cells tested with a monoclonal antibody specific for feline parvovirus. Canine parvovirus antigen is detected but no feline parvovirus is detected in the cells inoculated with the vaccine.

TESTS

Safety. Use 2 dogs of the minimum age recommended for vaccination and having no haemagglutination-inhibiting antibodies against canine parvovirus. Administer 10 doses of the vaccine to each dog by a recommended route. Observe for 14 days. No abnormal local or systemic reaction occurs.

Extraneous viruses. Mix the vaccine with a suitable antisera to canine parvovirus and inoculate into cell cultures known for their susceptibility to viruses pathogenic for the dog. No cytotoxic effect develops. There is no sign of haemagglutinating or haemadsorbing agents and no other sign of the presence of extraneous viruses.

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).