remaining animals as controls. 25-28 days after the last vaccination, infect all the animals by the conjunctival and/or intraperitoneal route with a suitable quantity of a virulent strain of the relevant *L. interrogans* serovar. Observe the animals for a further 28 days. Examine the dogs daily and record and score clinical signs observed post-challenge and any deaths that occur. If an animal shows marked signs of disease, it is killed. Monitor body temperatures each day for the first week after challenge. Collect blood samples from each animal on days 0, 2, 3, 4, 5, 8 and 11 post challenge. Collect urine samples from each animal on days 0, 3, 5, 8, 11, 14, 21 and 28 post challenge. Kill surviving animals at the end of the observation period. Carry out post-mortem examination on any animal that dies during the observation period and on the remainder when killed at the end of the observation period. In particular, examine the liver and kidneys for macroscopic and microscopic signs of leptospira infection. A sample of each kidney is collected and each blood, urine and kidney sample is tested for the presence of challenge organisms by re-isolation or by another suitable method. The blood samples are also analysed to detect biochemical and haematological changes indicative of infection and these are also scored.

The test is invalid if: samples give positive results on day 0; *L. interrogans* serovar challenge strain is re-isolated from or demonstrated by another suitable method to be present in fewer than 2 samples on fewer than 2 different days, to show infection has been established in fewer than 80 per cent of the control animals.

The vaccine complies with the test if: at least 80 per cent of the vaccinates show no more than mild signs of disease (for example, transient hyperthermia) and, depending on the *L. interrogans* serovar used for the challenge, one or more of the following is also shown:

- where the vaccine is intended to have a beneficial effect against clinical signs, the clinical scores and haematological and biochemical scores are statistically lower for the vaccinates than for the controls,
- where the vaccine is intended to have a beneficial effect against infection, the number of days that the organisms are detected in the blood is statistically lower for the vaccinates than for the controls,
- where the vaccine is intended to have a beneficial effect against urinary tract infection and excretion, the number of days that the organisms are detected in the urine and the number of kidney samples in which the organisms are detected is statistically lower for the vaccinates than for the controls.

LABELLING
The label states:
- the serovar(s) used to prepare the vaccine,
- the serovar(s) against which the protection is claimed.

**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 (5.2.6), absence of increase in virulence and immunogenicity (5.2.7).

**Safety**. The test is carried out for each route of administration stated on the label. Use not fewer than 5 susceptible puppies of the recommended minimum age for vaccination and that do not have antibodies against parainfluenza virus of canine origin. Administer to each puppy 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 puppies for 21 days. The puppies remain in good health and there is no abnormal local or systemic reaction.

If the vaccine is intended for use in pregnant bitches, administer the virus to not fewer than 5 bitches at the recommended stage or stages of pregnancy and according to the recommended schedule. Prolong the observation period until 1 day after whelping. The dogs 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**. Administer intranasally and by a recommended route to each of 2 puppies, 5 to 7 weeks old and which do not have antibodies against parainfluenza virus of canine origin, 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 in a batch of the vaccine. Collect nasal swabs from each dog daily from 3 to 10 days after inoculation. Inoculate the suspension from the swabs into suitable cell cultures to verify the presence of virus. Use the suspension from the swabs that contain the maximum amount of virus and administer intranasally 1 ml of the suspension into each of 2 other puppies of the same age and susceptibility. This operation is then repeated at least 5 times. If the virus is not recovered at a given passage level, a second series of passages is carried out. Inoculate virus from the highest recovered passage level to not fewer than 5 puppies, observe for 21 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.

**Immunogenicity**. The test described under Potency is suitable to demonstrate the 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.

**IDENTIFICATION**

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

**TESTS**

**Safety**. Use 2 puppies not older than the minimum age recommended for vaccination and which do not have antibodies against parainfluenza virus of canine origin. Administer a volume containing 10 doses of the vaccine into
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 inactivate 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 the quantity of 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.

**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**

Carry out test A or test B.

A. Inject subcutaneously into each of 5 guinea-pigs, free from specific antibodies, half of the dose stated on the label. 14 days after injection again half of the dose stated on the label. 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 liquid 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 × 10⁶ cells per millilitre. Allow to stand at 4 °C for 90 min and note the last dilution of serum that still completely inhibits haemagglutination.

**DEFINITION**

Inactivated canine parvovirus 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 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.