The vaccine complies with the test if there is no evidence of haemagglutinating activity and if not more than 20 per cent of the embryos die at either stage. If more than 20 per cent of the embryos die at one of the stages, repeat that stage; the vaccine complies with the test if there is no evidence of haemagglutinating activity and not more than 20 per cent of the embryos die at that stage. Antibiotics may be used in the test to control extraneous bacterial infection.

**Extraneous agents.** Inject a double dose by a recommended route into each of ten chickens, 14 to 28 days old and from a flock free from specified pathogens (5.2.2). After 3 weeks, inject one dose by the same route. Collect serum samples from each chicken 2 weeks later and carry out tests for antibodies against the following agents by the methods prescribed for chicken flocks free from specified pathogens (5.2.2): avian encephalomyelitis virus, avian infectious bronchitis virus, avian leucosis viruses, egg-drop syndrome virus, avian bursal disease virus, avian infectious laryngotracheitis virus, influenza A virus, Marek's disease virus. The vaccine does not stimulate the formation of antibodies against these agents.

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

**Potency**

Use two groups each of not fewer than twenty turkeys free from antibodies against avian paramyxovirus 3. Vaccinate one group in accordance with the recommendations for use. Keep the other group as controls. The test is invalid if serological tests carried out on serum samples obtained at the time of first vaccination show the presence of antibodies against avian paramyxovirus 3 in either vaccinates or controls or if tests carried out at the time of challenge show such antibodies in controls. At the egg-production peak, challenge the two groups by the oculo-nasal route with a suitable strain of avian tenosynovitis virus (avian orthoreovirus). This monograph applies to vaccines intended for administration to chickens for active immunisation.

**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.

2-4-1. **Safety.** Carry out the test for each route and method of administration to be recommended for vaccination using in each case chickens not older than the youngest age to be recommended for vaccination. 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, from an SPF flock (5.2.2). 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. Observe the chickens at least daily for 21 days. Carry out histological examination of the joints and tendon sheaths of the legs and feet at the end of the observation period (as a basis for comparison in the test for increase in virulence). The test is not valid if more than 10 per cent of the chickens die from causes not attributable to the vaccine virus. The vaccine virus complies with the test if no chicken shows notable clinical signs of avian viral tenosynovitis or dies from causes attributable to the vaccine virus.

2-4-2. **Increase in virulence.** The test for increase in virulence consists of the administration of the vaccine virus at the least attenuated passage level that will be present between the master seed lot and a batch of the vaccine to a group of five 1-day-old chicks from an SPF flock (5.2.2), sequential passages, 5 times where possible, to further groups of 1-day-old chicks and testing of the final recovered virus for increase in virulence. If the properties of the vaccine virus allow sequential passage to 5 groups via natural spreading, this method may be used, otherwise passage as described below is carried out and the maximally passaged virus that has been recovered is tested for increase in virulence. Care must be taken to avoid contamination by virus from previous passages. Administer by a suitable route a quantity of the vaccine virus that will allow recovery of virus for the passages described below. Kill the chickens at the moment when the virus concentration in the most suitable material (for example, tendons, tendon sheaths and liquid exudates from the hock joints, spleen) is sufficient. Prepare a suspension of this material from each chicken and pool these samples. Administer 0.1 ml of the pooled samples by the route of administration most likely to lead to increase in virulence to each of 5 other chickens of the same age and origin. Carry out this passage operation not fewer than 5 times; verify the presence of the virus at each passage. If the virus is not found at a passage level, carry out a second series of passages. Carry out the test for safety (section 2-4-1) using the unpassaged vaccine virus and the maximally passaged vaccine virus that has been recovered. The vaccine virus complies with the test if no indication of increase in virulence of the maximally passaged virus compared with the unpassaged virus is observed. If the virus is not recovered at any passage level in the first and second series of passages, the vaccine virus also complies with the test.
2-4-3. **Immunogenicity.** A test is carried out for each route and method of administration to be recommended using in each case chickens not older than the youngest age to be recommended for vaccination. The quantity of the vaccine virus administered to each chicken is not greater than the minimum virus titre to be stated on the label and the virus is at the most attenuated passage level that will be present in a batch of the vaccine. Use not fewer than 30 chickens of the same origin and from an SPF flock (5.2.2). Administer the vaccine by a recommended route to not fewer than 20 chickens. Maintain not fewer than 10 chickens as controls. Challenge each chicken after 21 days by a suitable route with a sufficient quantity of virulent avian tenosynovitis virus. Observe the chickens at least daily for 21 days after challenge. Record the deaths and the surviving chickens that show clinical signs of disease. If the challenge is administered by the foot pad, any transient swelling of the foot pad during the first 5 days after challenge may be considered non-specific. At the end of the observation period, kill all the surviving chickens and carry out macroscopic and/or microscopic examination for lesions of the joints and tendon sheaths of the legs and feet, e.g. exudate and swelling. The test is not valid if:

- during the observation period after challenge fewer than 80 per cent of the control chickens die or show severe clinical signs of avian viral tenosynovitis or show macroscopical and/or microscopic lesions in the joints and tendon sheaths of the legs and feet,
- or if during the period between 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 during the observation period after challenge not fewer than 90 per cent of the vaccinated chickens survive and show no notable clinical signs of disease or show macroscopical and/or microscopic lesions in the joints and tendon sheaths of the legs and feet.

3. **BATCH TESTS**

3-1. **Identification.** Carry out an immunostaining test in cell cultures to identify the vaccine virus.

3-2. **Bacteria and fungi**

Vaccines intended for administration by injection comply with the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062).*

Vaccines not intended for administration by injection either comply with the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)* or with the following test: carry out a quantitative test for bacterial and fungal contamination; carry out identification tests for microorganisms detected in the vaccine; the vaccine does not contain pathogenic microorganisms and contains not more than 1 non-pathogenic microorganism per dose.

Any liquid supplied with the vaccine complies with the test 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 of 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.** Titrate the vaccine virus by inoculation into suitable cell cultures (5.2.4). The vaccine complies with the test if 1 dose contains 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 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.

01/2005:1939

**BOVINE LEPTOSPIROSIS VACCINE (INACTIVATED)**

Vaccinum leptospirosis bovinae inactivatum

**DEFINITION**

Bovine leptospirosis vaccine (inactivated) is a suspension of inactivated whole organisms and/or antigenic extract(s) of one or more suitable strains of one or more of *Leptospira borgpetersenii* serovar hardjo, *Leptospira interrogans* serovar hardjo or other *L. interrogans* serovars, inactivated and prepared in such a way that adequate immunogenicity is maintained. This monograph applies to vaccines intended for active immunisation of cattle against leptospirosis.

**PRODUCTION**

The seed material is cultured in a suitable medium; each strain is cultivated separately. During production, various parameters such as growth rate are monitored by suitable methods; the values are within the limits approved for the particular product. Purity and identity are verified on the harvest using suitable methods. After cultivation, the bacterial harvest is inactivated by a suitable method. The antigen may be concentrated. The vaccine may contain an adjuvant.

**CHOICE OF VACCINE COMPOSITION**

The vaccine is shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) in cattle. As part of the studies to demonstrate the suitability of the vaccine with respect to these characteristics the following tests may be carried out.

**Safety**

A. The test is carried out for each route of administration to be stated on the label and in animals of each category (for example, young calves, pregnant cattle) for which the vaccine is intended. For each test, use not fewer than 10 animals that do not have antibodies against *L. borgpetersenii* serovar hardjo and the principal serovars of *L. interrogans* (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis). Use a batch of vaccine containing not less than the maximum potency that may be expected in a batch of vaccine. Administer to each animal a double dose of vaccine. If the recommended schedule requires a second dose, administer 1 dose after the recommended interval. Observe the animals for at least 14 days after the last administration. Record body temperatures the day before each vaccination, at vaccination, 4 h later and daily for 4 days. If the vaccine is intended for use or may be used in pregnant cattle,