B. For vaccine prepared with cell-culture-adapted strains of virus, inoculate ten doses of the vaccine into suitable cell cultures. If the vaccine contains an oil adjuvant, eliminate it by suitable means. Incubate at 38 ± 1 °C for 7 days. Make a passage on another set of cell cultures and incubate at 38 ± 1 °C for 7 days. The cultures show no signs of infection.

C. For vaccine prepared with strains of virus not adapted to embryos or cell cultures, inject two doses intramuscularly into each of twenty 14- to 28-day-old chickens from flocks free from specified pathogens (SPF) from each chicken. Pool the bursae and homogenise in an equal volume of a suitable liquid. Inject 1 ml of the bursal homogenate into each of a further ten chickens of the same age and from the same source. Examine microscopically the bursa of Fabricius of each remaining chicken from the first group and of each chicken from the second group 21 days after the injection; there is no evidence of infectious avian bursal disease infection. In addition there are no signs of any disease attributable to the vaccine and no abnormal local reaction develops. The chickens of the second group do not have antibodies against infectious avian bursal disease virus when examined 21 days after the injection.

Extraneous agents. Collect serum samples from each of the birds used in the safety test or inactivation test C, three weeks after vaccination. Carry out tests for antibodies to the following agents by the methods prescribed for chicken flocks free from specified pathogens (5.2.2): avian encephalomyelitis virus, avian leucosis viruses, haemagglutinating avian adenovirus, infectious bronchitis virus, infectious laryngotracheitis virus, influenza A virus, Marek’s disease virus, Newcastle 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

Vaccine each of not fewer than ten chickens, 4 weeks of age and from a flock free from specified pathogens (5.2.2). 4 days later, kill ten of the chickens and remove the bursa of Fabricius from each chicken. Pool the bursae and homogenise in an equal volume of a suitable liquid. Inject 1 ml of the bursal homogenate into each of a further ten chickens of the same age and from the same source. Examine microscopically the bursa of Fabricius of each remaining chicken from the first group and of each chicken from the second group 21 days after the injection; there is no evidence of infectious avian bursal disease infection. In addition there are no signs of any disease attributable to the vaccine and no abnormal local reaction develops. The chickens of the second group do not have antibodies against infectious avian bursal disease virus when examined 21 days after the injection.

Extraneous agents. Collect serum samples from each of the birds used in the safety test or inactivation test C, three weeks after vaccination. Carry out tests for antibodies to the following agents by the methods prescribed for chicken flocks free from specified pathogens (5.2.2): avian encephalomyelitis virus, avian leucosis viruses, haemagglutinating avian adenovirus, infectious bronchitis virus, infectious laryngotracheitis virus, influenza A virus, Marek’s disease virus, Newcastle 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

Vaccine each of not fewer than ten chickens, 4 weeks of age and from a flock free from specified pathogens (5.2.2), with one dose of vaccine by one of the recommended routes. 4 to 6 weeks later, collect serum samples from each bird and ten unvaccinated control birds of the same age and from the same source. Measure the antibody response in a serum-neutralisation test. Include in the test a suitable standard, calibrated in Ph.Eur. Units against infectious avian bursal disease serum BRP. The mean antibody level in the sera from the vaccinated chickens is not less than 10 000 Ph. Eur. Units per millilitre. There are no antibodies in the sera of the unvaccinated birds.

LABELLING

The label states whether the strain in the vaccine is embryo-adapted or cell-culture-adapted.
the master seed lot and a batch of the vaccine. Use not fewer than 20 chickens from an SPF flock \((5.2.2)\). Administer to each chicken a quantity of the vaccine virus equivalent to 10 times the maximum titre likely to be contained in a dose of the vaccine. On each of days 7, 14, 21 and 28 after administration of the vaccine virus, kill not fewer than 5 chickens and prepare a section from the site with the greatest diameters of the bursa of Fabricius of each chicken. Carry out histological examination of the section and score the degree of bursal damage using the following scale.

- **0** No lesion, normal bursa.
- **1** 1 per cent to 25 per cent of the follicles show lymphoid depletion (i.e. less than 50 per cent depletion in 1 affected follicle) influx of heterophils in lesions.
- **2** 26 per cent to 50 per cent of the follicles show nearly complete lymphoid depletion (i.e. more than 75 per cent depletion in 1 affected follicle), affected follicles show necrosis and severe influx of heterophils may be detected.
- **3** 51 per cent to 75 per cent of the follicles show lymphoid depletion; affected follicles show necrosis and severe influx of heterophils is detected.
- **4** 76 per cent to 100 per cent of the follicles show nearly complete lymphoid depletion, hyperplasia and cyst structures are detected; affected follicles show necrosis and severe influx of heterophils is detected.
- **5** 100 per cent of the follicles show nearly complete lymphoid depletion; complete loss of follicular structure, thickened and folded epithelium, fibrosis of bursal tissue.

Calculate the average score for each group of chickens. The vaccine virus complies with the test if:

- no chicken shows notable clinical signs of disease or dies from causes attributable to the vaccine virus,
- the average score for bursal damage 21 days after administration of the vaccine virus is less than or equal to 2.0 and 28 days after administration is less than or equal to 0.6,
- during the 21 days after administration a notable repopulation of the bursae by lymphocytes has taken place.

**2-4-3. Immunosuppression.** Carry out the tests for the route recommended for vaccination likely to be the least safe using chickens not older than the youngest age 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. Use not fewer than 30 chickens from an SPF flock \((5.2.2)\). Divide them randomly into 3 groups each of not fewer than 10 and maintain the groups separately. Administer by eye-drop to each chicken of 1 group a quantity of the vaccine virus equivalent to not less than the maximum titre likely to be contained in 1 dose of the vaccine. At the time after administration when maximal bursal damage is likely to be present, as judged from the results obtained in the test for damage to the bursa of Fabricius \((section ~2-4-2)\), administer to each vaccinated chicken and to each chicken of another group 1 dose of Hitchner B1 strain Newcastle disease vaccine \((live)\). Determine the seroresponse of each chicken of the 2 groups to the Newcastle disease virus 14 days after administration. Challenge each chicken of the 3 groups by the intramuscular route with not less than \(10^6 \text{EID}_{50}\) of virulent Newcastle disease virus and note the degree of protection in the 2 groups vaccinated with Hitchner B1 strain Newcastle vaccine compared with the non-vaccinated group. The test is not valid if 1 or more of the non-vaccinated chickens does not die within 7 days of challenge. The degree of immunosuppression is estimated from the comparative seroresponses and protection rates of the 2 Hitchner B1 vaccinated groups. The vaccine complies with the test if there is no significant difference between the 2 groups.

**2-4-4. 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 5 chickens from an SPF flock \((5.2.2)\) and not older than the youngest age to be recommended for vaccination, sequential passages, 5 times where possible, to further similar groups 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 eye-drop a quantity of the vaccine virus that will allow recovery of virus for the passages described below. Prepare 3 to 4 days after administration a suspension from the bursa of Fabricius of each chicken and pool these samples. Administer 0.05 ml of the pooled samples by eye-drop 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 damage to the bursa of Fabricius \((section ~2-4-2)\) using the unpassaged vaccine virus and the maximally passaged virus that has been recovered. Administer the virus by the route recommended for vaccination likely to be the least safe. The vaccine virus complies with the test if no indication of increasing virulence of the maximally passaged virus compared with the unpassaged virus is observed. If virus is not recovered at any passage level in the first and second series of passages, the vaccine virus also complies with the test.

**2-4-5. 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 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 \((5.2.2)\). Vaccinate by a recommended route not fewer than 20 chickens. Maintain not fewer than 10 chickens as controls. Challenge each chicken after 14 days by eye-drop with a sufficient quantity of virulent avian infectious bursal disease virus. Observe the chickens at least daily for 10 days after challenge. Record the deaths due to infectious bursal disease and the surviving chickens that show clinical signs of disease. At the end of the observation period, kill all the surviving chickens and carry out histological examination for lesions of the bursa of Fabricius. The test is not valid if 1 or more of the following applies:

- during the observation period following challenge, fewer than 50 per cent of the control chickens show characteristic signs of avian infectious bursal disease,
- 1 or more of the surviving control chickens does not show degree 3 lesions of the bursa of Fabricius,
Avian infectious encephalomyelitis vaccine (live)

Vaccinum encephalomyelitidis infectivae aviariae vivum

1. DEFINITION
Avian infectious encephalomyelitis vaccine (live) is a preparation of a suitable strain of avian encephalomyelitis virus. This monograph applies to vaccines intended for administration to non-laying breeder chickens to protect passively their future progeny and/or to prevent vertical transmission of virus via the egg.

2. PRODUCTION

2-1. PREPARATION OF THE VACCINE
The vaccine virus is grown in embryonated hens' eggs or in cell cultures.

2-2. SUBSTRATE FOR VIRUS PROPAGATION

2-2-1. Embryonated hens' eggs. If the vaccine virus is grown in embryonated hens' eggs, they are obtained from flocks free from specified pathogens (SPF) (5.2.2).

2-2-2. Cell cultures. If the vaccine virus is grown in cell cultures, they comply with the requirements for cell cultures for production of veterinary vaccines (5.2.4).

2-3. SEED LOTS

2-3-1. Extraneous agents. The master seed lot complies with the tests for extraneous agents in seed lots (2.6.24). In these tests on the master seed lot, the organisms used are not more than 5 passages from the master seed lot at the start of the tests.

2-4. 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.

The following tests for safety (section 2-4-1), increase in virulence (section 2-4-2) and immunogenicity (section 2-4-3) may be used during the demonstration of safety and immunogenicity.

2-4-1. Safety. Carry out the test for each route and method of administration to be recommended for vaccination using in each case non-laying breeder 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 equivalent to 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. The test is not valid if more than 10 per cent of the chickens show abnormal clinical signs or die from causes not attributable to the vaccine.

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 vaccine to a group of five 1-day-old chickens from an SPF flock (5.2.2), sequential passages, 5 times where possible, to further

3. BATCH TESTS

3-1. Identification. The vaccine, diluted if necessary and mixed with a monospecific infectious bursal disease virus type 1 antiserum, no longer infects embryonated hens' eggs from an SPF flock (5.2.2) or susceptible cell cultures (5.2.4) into which it is inoculated.

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 embryonated hens' eggs from an SPF flock (5.2.2) or 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 2-4-5) 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.