Avian Infectious Bronchitis Vaccine, Inactivated

Avian Infectious Bronchitis Vaccine, Inactivated consists of an emulsion or a suspension of one or more serotypes of avian infectious bronchitis virus which have been inactivated in such a manner that the immunogenic activity is retained. This monograph applies to vaccines intended to protect birds against drop in egg production or quality; for vaccines also intended for protection against respiratory signs and nephropathic symptoms, a demonstration of efficacy additional to that described under potency is required.

Production

The virus is propagated in embryonated hen’s eggs obtained from healthy flocks or in suitable cell culture derived from SPF eggs (2.7.7). The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10). The vaccine may contain one or more suitable adjuvant.

Inactivation

An amplification test for residual live avian infectious bronchitis virus is carried out on each batch of antigen immediately after inactivation. The test is carried out in fertilised hen’s eggs from flocks free from specified pathogens (SPF) or in suitable cell culture derived from SPF eggs (2.7.7)s and the quantity of inactivated virus used is equivalent to not less than 10 doses of vaccine. No live virus is detected.

A. In embryonated eggs: For vaccine prepared with embryo-adapted strains of virus, inject quantity of inactivated virus equivalent to 10 doses of vaccine into the allantoic cavity of ten 9 to 11-day-old fertilized hen eggs from an SPF flock and incubate. Observe for 5 to 6 days and pool separately the allantoic fluid from eggs containing live embryos and that from eggs containing dead embryos, excluding those that die within the first 24 hours after injection. Examine for abnormalities in all embryos which die after 24 hours of inoculation or which survive 5 to 6 days. No death or abnormality attributable to the vaccine virus occurs.

Inject into the allantoic cavity of each of ten 9 to 11-day-old fertilized hen eggs from SPF flock, 0.2 ml of the pooled allantoic fluid from the live embryos and into each of 10 similar eggs 0.2 ml of the pooled liquid from the dead embryos and incubate for 5 to 6 days. Examine for abnormalities all embryos which die after 24 hours of injection or which survive 5 to 6 days. No death or abnormality attributable to the vaccine virus occurs.

If more than 20 per cent of the embryos die at either stage repeat the test from that stage. The vaccine complies with the test if there is no death or abnormality attributable to the vaccine virus. Antibiotics may be used to control extraneous bacterial infection

B. In Cell culture: For vaccine prepared with cell-culture-adapted strains of virus, inoculate quantity of inactivated virus equivalent to 10 doses of vaccine into suitable cell culture derived from SPF eggs (2.7.7)s. Incubate at 36± 1° for 7 days. Make a passage on another set of cell culture derived from SPF eggs (2.7.7) and incubate at 36 ± 1° for 7 days. None of the cultures shows signs of infection.

Identification

In susceptible birds, the vaccine stimulates the production of specific antibodies against each of the virus strain incorporated in the vaccine, detectable by suitable serological method.

Tests

Sterility(2.2.11). Complies with the test for sterility.

Safety. Inject intramuscularly a quantity equivalent to 2 doses into each of ten SPF chickens (2.7.7) or healthy susceptible chickens, 2 to 4 weeks old. Observe the chickens for 14 days. No abnormal systemic or local reaction is seen.
**Potency.** Inject one dose by the route stated on the label into each of 10 SPF chickens (2.7.7 table 3) or healthy susceptible chickens, 3 to 4 weeks old. Use 5 similar chickens as controls and house them together with the vaccinated chickens. After 28 days, collect serum samples from each of the vaccinated and control chickens and perform haemagglutination inhibition (HI) test on each serum using 4 haemagglutinating (HA) units of antigen and chicken erythrocytes, testing all serum samples at the same time. The vaccine passes the test if the mean antibody titre of the vaccinated group is not less than 1:64 and no specific antibody is detected in the control chickens. Alternatively, serum neutralization test may be carried out in SPF eggs (2.7.7). Serum neutralization titer should not be less than $10^2$ neutralization units.

**Storage.** When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

**Labelling.** The label states (1) the strain of virus used in preparing the vaccine; (2) route of administration.
Avian Infectious Bronchitis Vaccine, Live

Synonyms: Infectious Bronchitis Vaccine, Live; Avian Infectious Bronchitis Vaccine Living

Avian Infectious Bronchitis Vaccine, live is a preparation of one or more suitable strains of avian infectious bronchitis virus.

**Production**
The vaccine virus is grown in embryonated hens’ eggs or in cell culture derived from SPF eggs (2.7.7).

**Substrate for virus propagation**
If the vaccine virus is grown in embryonated hen’s eggs they are obtained from SPF flock (2.7.7) or in cell culture derived from SPF flocks (2.7.7).

The production is based on an approved seed lot system. Each lot of stock seed virus is tested for immunogenicity in chicken of the same age and source by the method described under immunogenicity test. If the immunogenicity test has been performed with satisfactory results on the representative batch of vaccine from the seed lot, it may be omitted as a routine control of other batches of the vaccine prepared from the same seed lot.

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

**Identification**
Carry out either the test A or B.

A. Inoculate 0.2 ml undiluted vaccine in the allantoic sac of SPF embryonated eggs and incubate at 36 ± 1°C for 5 to 6 days. Lesions typical of infectious bronchitis are observed in the embryos and the allantoic fluid does not agglutinate chicken erythrocytes.

B. Specific antiserum against the strain or each of the strains of the avian infectious bronchitis virus used in the vaccine should neutralise corresponding IB virus. When mixed with specific antiserum, the vaccine no longer infects 9-11 day old embryonated SPF eggs (2.7.7).

**Tests**

**Moisture** (2.3.43). Not more than 3.0 per cent.

**Mycoplasmas** (2.7.9). Complies with the test for mycoplasmas.

**Safety.** Inject 10 times the dose by the route stated on the label into each of 10 SPF chickens (2.7.7 table 3) or healthy susceptible chickens of 5-10 days old. Observe the birds for 21 days. Not more than one of the vaccinated chickens shows symptoms of or dies from infectious bronchitis. If during the period of observation more than 2 of the vaccinated chickens die from causes not attributable to the vaccine, repeat the test.

**Sterility** (2.2.11). Complies with the test for sterility.

**Virus titre.** Titrate the vaccine in cell culture derived from SPF eggs (2.7.7) derived from SPF embryos or by inoculating into the allantoic sac of SPF embryonated eggs, 9 to 11 days old. One dose of the vaccine contains not less than $10^{3.5}$ TCID$_{50}$/EID$_{50}$.

**Immunogenicity:** Carry out a test for each route of administration recommended on the label and for each serotype against which protection is claimed and of the minimum age stated for vaccination. Administer to each of 20 SPF chickens (2.7.7 table 3) or healthy susceptible chickens, 3 to 4 weeks old, for each of the...
stated routes a volume of reconstituted vaccine containing a quantity of virus equivalent to the minimum titre stated on the label. Ten additional SPF chickens (2.7.7 table 3) or healthy susceptible chickens of same flock for each serotype against which protection is claimed are used as unvaccinated controls. Three to four weeks later, administer by eye drop a virulent strain of bronchitis virus with a titre of at least $10^{3.5}$ EID$_{50}$ per ml to all the vaccinated and control birds. Between the fourth to seventh day after the challenge, take tracheal swabs from each of the vaccinated and control birds. Place each swab in a sterile test tube containing 3 ml of tryptose phosphate broth and antibiotics. Swirl the tubes containing swabs thoroughly and store at −20°C pending inoculation into eggs. For each tracheal swab, inoculate at least 5 chicken embryos, 9 to 11 days old, with 0.2 ml of the broth from each tube into the allantoic cavity. All the embryos surviving on the third day after inoculation are used in the evaluation. A tracheal swab is considered positive for recovery of the virus if any of the embryos shows typical infectious bronchitis lesions such as stunting, curling, kidney urates, clubbing down or death between the fourth and seventh day after inoculation. The vaccine complies with the test if not less than 80 per cent of the controls and not more than 20 per cent of the vaccinated chickens are positive for virus recovery. If less than 80 per cent of the vaccinated chickens are negative for virus recovery the stock seed is unsatisfactory. The stock seed virus may be tested for immunogenicity once in 5 years provided it is maintained under standard conditions of storage of the bronchitis virus.

**Storage.** When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

**Labelling.** The label/Insert states (1) the minimum virus titre per dose; (2) Dose of vaccine.
Avian Spirochaetosis Vaccine

Avian Spirochaetosis vaccine is a suspension prepared from viscera and membranes of developing chicken embryos of SPF eggs (2.7.7) infected with antigenic strains of *Borrelia anserinea*, which has been inactivated in a such a manner that it’s immunogenic activity is retained.

**Production**

Substrate for propagation

The organism is grown in embryonated eggs derived from SPF flocks.

**Identification**

Protects chickens against infection with *B. anserina*.

**Tests**

**Safety.** Inject subcutaneously a quantity equivalent to 2 doses into each of 10 SPF chickens (2.7.7 table 3) or healthy susceptible chickens of the recommended age at which vaccine is to be used. Observe the chickens for 14 days; no abnormal systemic or local reaction is seen.

**Sterility** (2.2.11). Complies with test for sterility.

**Potency.** Inject at least 10 SPF chickens (2.7.7 table 3) or healthy susceptible chickens, 8 to 12 week old, with the minimum dose of vaccine by the route stated on the label. Use 5 chickens of the same stock as controls. Ten days later challenge all the chickens intra-peritoneally with an adequate dose of a virulent culture of *B. anserina* used to prepare the vaccine or with a suspension of liver or kidney tissues obtained from infected chickens. Observe the chickens for 10 days. The vaccinated chickens do not show any symptoms of the disease and presence of *B. anserina* organism in the blood smears of the vaccinated group. The test is not valid unless the control chickens show typical symptoms of Spirochaetosis with detection of spirochetes in the blood smears.

**Storage.** When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

**Labelling.** The label/Insert states (1) Strain of the bacterial used; (2) Route of administration.
Egg Drop Syndrome 76 (Adenovirus) Vaccine, Inactivated

Synonyms: Egg Drop Syndrome 76 (Adenovirus) Vaccine

Egg Drop Syndrome 76 (Adenovirus) Vaccine, Inactivated consists of an emulsion or a suspension of a suitable strain of egg drop syndrome ‘76 virus (haemagglutinating avian adenovirus) which has been inactivated in such a manner that immunogenic activity is retained.

Production

The vaccine strain is propagated in embryonated duck eggs from healthy flocks or in suitable cell culture derived from SPF eggs (2.7.7). The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Test for inactivation.

The test for inactivation is carried out in fertilized duck eggs from a flock free from egg drop syndrome ‘76 virus infection or hen eggs from a flock free from specified pathogens, or in suitable cell culture derived from SPF eggs (2.7.7), whichever is the most sensitive for the vaccine strain; the quantity of virus used in the test is equivalent to not less than ten doses of the vaccine. No live virus is detected.

The vaccine may contain suitable adjuvant.

Identification

When inoculated into chicken, the development of specific neutralizing antibodies against egg drop syndrome ‘76 (adenovirus) can be demonstrated by suitable serological tests.

Tests

Safety. Inject each of ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens between 2 and 4 weeks old, with two doses and by the route stated on the label. Observe the chicken for 14 days. None of the chicken shows any abnormal local or systemic reaction.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject each of twenty SPF chickens (2.7.7 table 3) or healthy susceptible chickens, 3 to 4 weeks old, with the dose and by the route stated on the label. After 21 days, collect serum samples from each of the birds as well as ten-control chickens of the same stock and perform haemagglutination inhibition test on each serum using 4 haemagglutinating units of antigen and chicken erythrocytes. The vaccine passes the potency test if the mean antibody titre of the vaccinated group is greater than 1:128. The test is valid only if no specific antibody is found in the control chicken.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

Labelling. The label/Insert states (1) the strain used for the preparation; (2) Route of administration.
Fowl Cholera Vaccine, Inactivated
Syonyms: Pasturella multocida vaccine for chickens
Fowl Cholera Vaccine, Inactivated is a preparation of suitable strains of 1 or more serovars of *Pasteurella multocida*. This monograph applies to vaccines intended for the active immunisation of chickens, turkeys, ducks and geese against fowl cholera Infection.

Production
The seed material is inoculated in a suitable medium. If the vaccine contains more than 1 strain of bacterium, the different strains are grown and harvested separately. The bacterial harvests are inactivated with suitable agent. The vaccine may contain suitable adjuvant.

Identification
Protects susceptible chicken against infection with *P. multocida*.

Tests
**Safety.** Administer double dose of vaccine subcutaneously into each of ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens of 4 to 6 weeks age. Observe the chickens for 7 days; none of the chicken shows untoward reaction other than slight transient local swelling.

**Sterility** (2.2.11). Complies with the test for sterility.

**Potency.** Immunize 20 SPF chickens (2.7.7 table 3) or healthy susceptible chickens 4 to 6 weeks of age per strain incorporated in the batch with one dose of vaccine. Give booster dose of vaccine 15 to 21 days after primary immunization. Keep unvaccinated healthy susceptible control birds of similar age 10 birds per strain incorporated in vaccine. Challenge the birds with an appropriate dose of virulent 18 hour old broth culture of recently bird passaged strain of *Pasteurella multocida* that shall kill at least 80% of the unvaccinated susceptible chickens. Observe birds for 14 days post challenge. There should be not less than 70% protection of vaccinated birds and specific mortality of at least 80% in control.

**Storage.** When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

**Labelling.** The label/Insert states (1) the serovar(s) used to prepare the vaccine; (2) Route of administration.
Fowl Pox / Pigeon Pox Vaccine, Live

Fowl Pox / Pigeon Pox Vaccine, Live is a preparation of a suitable strain / s of pigeon pox virus or fowl pox virus. This monograph applies to vaccines intended for administration to chickens for active immunization against avian pox virus.

Production

The vaccine virus is grown in embryonated hens’ eggs from SPF flock (2.7.7) or in cell cultures derived from SPF eggs (2.7.7) or cell lines. The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Substrate for virus propagation

The vaccine virus is grown either in embryonated hens’ eggs from flocks free from specified pathogens SPF (2.7.7) or in avian cell cultures obtained from flocks free from specified pathogens SPF (2.7.7) or cell lines.

Identification

Carry out an immunostaining or neutralization test in cell culture derived from SPF eggs (2.7.7) to demonstrate the presence of the vaccine virus or inoculate the vaccine into eggs and notice the characteristic lesions.

Tests

Moisture (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). The vaccine complies with the test for mycoplasmas.

Safety. Administer 10 doses of the vaccine to each of ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens 6 to 8 weeks old by the route stated on the label. Observe the birds for 21 days. No chicken dies from causes attributable to the vaccine or shows signs of toxicity other than mild, transient, local reactions. If during the observation period more than two chickens die from causes not attributable to the vaccine, repeat the test.

Virus titre. Not less than $10^2$ EID$_{50}$/TCID$_{50}$ of the virus per dose, determining the titre by inoculation into the chorio-allantoic membrane of SPF embryonated eggs, between 9-11 days old, or one or more route for virus titration depending upon the strain.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out a separate potency test for each of the routes of administration stated on the label. Use not less than ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens, 6 to 8 weeks old. Use ten birds from the same flock and weight range as controls. Administer to each chicken a volume of the reconstituted vaccine containing a quantity of the virus equivalent to the minimum titer stated on the label. After 21 days, challenge each chicken by intrafollicular administration or by scarification with a virulent strain of fowl pox virus. Observe the birds for 14 days. The vaccinated chickens survive and show no signs of disease except transient local reactions of fowl pox within 6 days following the challenge. All control chickens show lesions of fowl pox.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine it may be omitted as a routine test during production of the other batches of the vaccine prepared from the same seed lot.
Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

Labelling. The label/Insert states (1) the minimum virus titre; (2) Dose of vaccine.
Inclusion Body Hepatitis (IBH) Vaccine, Inactivated

Inclusion Body Hepatitis (IBH)/Hydropericardium Syndrome (HPS) Vaccine (Inactivated) consists of an emulsion or a suspension of avian adenovirus(es) which have been inactivated in such a manner that the immunogenic activity is retained. The vaccine may contain one or more suitable adjuvants.

Production

Substrate for virus propagation

Vaccine virus is multiplied in healthy susceptible chicks or SPF eggs (2.7.7) or in cell culture derived from SPF eggs (2.7.7).

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Test for Inactivation

To confirm inactivation an amplification test for residual live IBH/HPS virus is carried out on each batch of antigen immediately after inactivation or on the final bulk (if the vaccine contains a mixture of inactivated antigens). The test is conducted on healthy susceptible chickens demonstrated to be free from antibodies to IBH/HPS virus or in fertilized eggs derived from specific pathogen free flocks (2.7.7) if the vaccine virus has been propagated in embryos. The quantity of inactivated virus used in the test is equivalent to not less than ten doses of the vaccine. No live virus is detected.

Identification

Protects chickens against infection of IBH/HPS.

Tests

Safety. Inject subcutaneously a quantity equivalent to 2 doses into each of 10 SPF chickens (2.7.7 table 3) or healthy susceptible chickens of the recommended age at which vaccine is to be used. Observe the chickens for 14 days, no abnormal systemic or local reaction is seen.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Either test A or Test B may be carried out.

A. Inject one dose by the route stated on label into each of 20 SPF chickens (2.7.7 table 3) or healthy susceptible chickens at the age recommended by manufacturer. Use 10 similar chickens from same source as unvaccinated controls. After 10 days of immunization challenge the birds with 10% IBH positive infected liver suspension 0.5 ml per bird. Observe the birds for ten days. The vaccine passes the potency test when there is 90% protection in vaccinated bird and 80% deaths in unvaccinated controls.

B. At least five 3-6 week old SPF chickens (2.7.7 table 3) or healthy susceptible chickens are vaccinated with one field dose of vaccine by intramuscular route. Blood samples are collected between 3 and 5 weeks and the antibody response measured by ELISA. The mean antibody titer should be at least 10 log₂ ELISA units.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.
Labelling. The label/Insert states (1) strain used for vaccine production; (2) Route of administration.


**Infectious Avian Encephalomyelitis Vaccine, Live**

**Synonyms:** encephalomyelitis Vaccine Live, Epidemic Tremor Vaccine Live

Infectious Avian Encephalomyelitis Vaccine, Live is a freeze-dried preparation of an attenuated strain of infectious avian encephalomyelitis virus.

**Production**

The virus is grown in SPF embryonated eggs (2.7.7) or in suitable cell culture derived from SPF eggs (2.7.7). The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

**Identification**

Inoculate 0.1 ml of the undiluted reconstituted vaccine into the yolk sac of SPF embryonated eggs, between 5 to 6 days old. Keep them in an incubator and transfer to the setter for hatching. Observe the hatched chickens for 7 days. Not less than 50 per cent of the chickens show the typical symptoms characteristic of infectious avian encephalomyelitis-like weakness or paralysis of legs, sitting posture on hock joints and tremors.

**Tests**

**Moisture** (2.3.43). Not more than 3.0 per cent.

**Mycoplasmas** (2.7.9). The vaccine complies with the test for mycoplasmas

**Safety.** Administer ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens by ten doses of the vaccine by the recommended route. Observe the chickens for 21 days. No chicken develops signs of the disease or dies from causes attributable to the vaccine. Repeat the test if more than two chickens die from causes not attributable to the vaccine during the observation period.

**Virus titre.** Not less than $10^{2.5}$ TCID$_{50}$/EID$_{50}$ of the virus per dose, determining the titre of the virus in cell culture derived from SPF eggs (2.7.7) or by inoculation into the yolk sac of SPF embryonated hen eggs (2.7.7), between 5 to 6 days old.

**Sterility** (2.2.11). Complies with the test for sterility.

**Potency.** Carry out a separate potency test for each of the routes of administration stated on the label. For each of the stated routes, use not less than ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens, 3 weeks old. Administer to each chicken a volume of the reconstituted vaccine containing a quantity of the virus equivalent to the minimum virus titre stated on the label. Use ten chickens of the same flock and age as controls. After 21 days, challenge each chicken in the vaccinated and control groups with intracerebral injection of a suitable quantity of a virulent avian encephalomyelitis virus. Observe the chickens for another 21 days. Not less than 80 per cent of the vaccinated chickens survive or show no signs of disease and not less than 70 per cent of the controls die or develop signs or paralytic lesions of avian encephalomyelitis.

If the potency test has been performed with satisfactory results on representative batch of the vaccine from the same seed lot, it may be omitted as a routine control test during production of other batches of the vaccine prepared from the same seed lot.

**Storage:** When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.
**Labelling.** The label/insert states (1) the minimum virus titre; (2) Dose of vaccine.
Infectious Bursal Disease Vaccine, Inactivated

Infectious Bursal Disease Vaccine, Inactivated consists of an emulsion or a suspension of a suitable strain of infectious bursal disease virus which has been inactivated in such a manner that immunogenic activity is retained. The vaccine may contain one or more suitable adjuvant.

Production

The virus is propagated in fertilized eggs obtained from healthy flock or in suitable cell culture derived from SPF eggs (2.7.7) or in healthy susceptible chicken.

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Inactivation

An amplification test for residual live infectious bursal disease virus is carried out on each batch of antigen immediately after inactivation and the test is carried out in fertilized hen eggs obtained from SPF flocks (2.7.7) or in suitable cell culture derived from SPF eggs (2.7.7) or, where chickens have been used for production of the vaccine, in chickens from a flock free from specified pathogens. The quantity of inactivated virus used in the test is equivalent to not less than ten doses of the vaccine. No live virus is detected.

Test For Inactivation

For vaccine prepared with embryo-adapted strains of the virus. Inject quantity of inactivated virus equivalent to 10 doses of vaccine into the allantoic cavity or onto the chorio-allantoic membrane of the SPF embryonated hen eggs, between 9 to 11 days old, and incubate at 36±1°. Observe for 6 days and pool separately the allantoic fluid from eggs containing live embryos, and that from eggs containing dead embryos, excluding those dying from non-specific causes within the first 24 hours after inoculation. Inject into the allantoic cavity of each of the SPF embryonated hen eggs, between 9 to 11 days old, 0.2 ml of the pooled allantoic fluid from the live embryos or membrane from the dead embryos and incubate at 36±1° for 6 days. Examine each embryo for lesions of infectious bursal disease. The vaccine complies with the test if there is no evidence of lesions of infectious bursal disease. The test is valid only if not more than 20 per cent of the embryos die at either stage of the test. If more than 20 per cent of the embryos die at either one of the stages of the test, repeat that stage. In any repeat test, not more than 20 per cent of the embryos die from non-specific causes. Antibiotics may be used to control extraneous bacterial infection.

For vaccine prepared with strains of virus not adapted to embryos. Inject two doses intramuscularly into each of twenty chickens, between 14 and 28 days old, complying with the requirements stated under Test on chicken flocks free from pathogens for the production and quality control vaccines (2.7.7). Four day later, kill ten of the chickens and remove bursa of fabricius from each chicken, pool the bursa and homogenise in an equal volume of a suitable liquid. Inject 1 ml of the homogenate into each of a further ten chickens of the same flock and age. After 21 days, examine microscopically the bursa of each chicken from the first group and the second group. No evidence of infectious bursal disease is seen and no abnormal local reaction develops.

For vaccine prepared with cell culture-adapted strains of the virus. The formaldehyde in the test sample is neutralized with sodium metabisulphite. Five ml is tested for the presence of infective Gumboro Disease virus by inoculation of at least 800 square cm primary or secondary CEF. The cultures are incubated for 3 to 4 days at a temperature of 37°. After one cycle of freezing and thawing the supernatant from these cultures is passaged onto a fresh CEF cultures. Three to four days latter this is repeated. Three to four days after final inoculation the cultures are inspected for CPE. A vital stain and overlay may be used. If no trace of CPE is detected, the inactivation of the antigen suspension is accepted to be completed.
Identification

Protects susceptible chickens against infectious bursal disease by producing specific antibodies on inoculation.

Tests

Safety. Inject each of ten healthy chickens, 14 to 28 days old with twice the minimum vaccinating dose and by one of the routes stated on the label. Observe the chickens for 14 days. No abnormal local or systemic reaction is seen.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject each of ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens, 3 to 4 weeks old, with the minimum dose and by the route stated on the label. Use ten chickens of the same flock and age as controls. After 21 days, collect serum samples from each bird including the ten-control chickens and perform quantitative agar gel precipitation test or serum neutralizing test on each serum sample. The mean antibody titer of sera in vaccinated group shall be 1:8 by Agar gel diffusion test and 10000 units per ml by serum neutralization test and there are no IBD specific antibodies in the sera of control chicken.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

Labelling. The label/Insert states (1) the type of strain; (2) Route of administration.
Infectious Bursal Disease Vaccine, Live

Infectious Bursal disease vaccine, live is a freeze dried preparation of attenuated strain of infectious bursal disease virus.

Production

Infectious Bursal Disease Vaccine, Live is a suitable strain of Infectious Bursal Disease virus. This monograph applies to vaccines intended for administration to chickens for active immunization. The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Substrate for Virus Propagation

The vaccine virus is grown in embryonated eggs obtained from SPF flocks or in cell culture derived from SPF eggs (2.7.7) or susceptible cell lines.

Identification

When mixed with monospecific infectious bursal disease virus antiserum the vaccine no longer infects susceptible cell culture derived from SPF eggs (2.7.7) or embryonated hen eggs, 9 to 11 days old.

Tests

Moisture (2.3.43). Not more than 3.0 per cent w/w.

Mycoplasmas (2.7.9). Vaccine Complies with test for mycoplasmas.

Safety. Use not fewer than ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens, 10 to 15 days old. According to the type of viral vaccine strain incorporated in the product - Invasive - Moderately invasive- it may be necessary to conduct the safety test on chicks possessing moderate level of maternal antibodies.

Administer by eye drop to each chicken ten doses of the vaccine reconstituted so as to obtain a concentration suitable for the test. Observe the chickens for 21 days. If during the period of observation more than 2 chickens die from causes not attributable to the vaccine, repeat the test. The vaccine complies with the test if no chickens shows signs of the disease, if no chicken dies from causes attributable to the vaccine and if 21 days after inoculation of the vaccine, no chicken shows lesions of the bursa of Fabricius.

Sterility (2.2.11). Complies with the test for sterility.

Virus titre. Not less than $10^2$ TCID$_{50}$/EID$_{50}$ of the virus per dose. Determining the titre in cell cultures derived from SPF embryo or onto the chorio-allantoic membrane of SPF embryonated hen eggs between 9-11 days old.

Potency. Use 20 SPF chickens (2.7.7 table 3) or healthy susceptible chickens 10 to 15 day old. Administer to each chicken one dose of the vaccine by recommended route. Use 10 chickens of the same flock and age as controls. Fourteen days after immunization challenge chicken of both groups, by intraocular route administration of a suitable quantity of virulent infectious bursal disease virus. Observe the birds for 10 days after challenge. Not more than 4 of vaccinated chickens die or show signs of the infectious bursal disease or on histological examination show severe bursal lesions. The test is not valid unless not less than 50% of the control birds die or show signs of IBD and all the surviving controls show severe bursal lesions on histological examination.
If at least 90% of the follicles show greater than 75% depletion of lymphocytes, the bird is considered as one showing severe bursal lesions.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

**Storage.** When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

**Labelling.** The label/Insert states (1) minimum virus titre; (2) dose of Vaccine.
Infectious Chicken Anemia Vaccine, Inactivated

Infectious Chicken Anemia vaccine, Inactivated is a preparation of a suitable strain of chicken anemia virus, inactivated in such a manner that the immunogenic activity is retained. This monograph applies to vaccines intended for administration to chickens for immunization.

Production

Substrate for Propagation
The vaccine is grown in embryonated hen’s egg obtained from SPF flocks or in suitable cell culture derived from SPF eggs (2.7.7) or susceptible cell line. Harvested virus is inactivated using suitable inactivating agent. The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10)

Inactivation
An amplification test for residual live chicken infectious anemia virus is carried out on each batch of antigen immediately after inactivation. The test is carried out in suitable cell culture derived from SPF eggs (2.7.7) or using susceptible cell lines. The quantity of inactivated virus used in the test is equivalent to not less than ten doses of the vaccine. No live virus is detected.

Test for Inactivation
Inoculate 10 doses of vaccine virus using suitable cell culture derived from SPF eggs (2.7.7) or in susceptible cell lines or SPF eggs (2.7.7). Incubate at 36 ±1⁰ for 7 days. Make a passage on another set of cell culture derived from SPF eggs (2.7.7) or in cell lines or embryonated SPF eggs (2.7.7) and incubate at 36 ±1⁰ for 7 days. None of the cultures shows signs of CPE.

Identification
In susceptible chicks, the vaccine stimulates the production of specific antibodies against vaccine virus detected by suitable serological tests.

Test

Safety. Inject a double dose of vaccine by recommended route in to each of ten, 14 to 28 day-old SPF chickens (2.7.7 table 3) or healthy susceptible chickens. Observe the chickens for 21 days. No abnormal local or systemic reactions occur.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out a potency test for the route of administration stated on the label. Vaccinate, ten, 21 to 28 day old SPF chickens (2.7.7 table 3) or healthy susceptible chickens with one dose of vaccine. Keep 10 unvaccinated birds of the same age group as controls. Observe the birds for 28 days. Collect serum samples from each bird including the ten-control chickens. Detect the virus specific antibodies by serological methods i.e. Enzyme Linked Immunoassay or Virus Neutralization test. The mean serum neutralization antibody titer of sera in vaccinated group shall be 5000 units per ml and there are no CAV specific antibodies in the sera of control chickens.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.
**Labelling.** The label/Insert states (1) strains used for preparation; (2) route of administration.
**Infectious Chicken Anemia Vaccine, Live**

Infectious chicken anemia vaccine (Live) is a preparation of a suitable strain of chicken anemia virus. This monograph applies to vaccines intended for administration to breeder chicken for active immunization, to prevent excretion of virus, to prevent or reduce transmission through eggs.

**Production**

Substrate for propagation

Vaccine is grown either in embryonated hen’s egg obtained from SPF flocks (2.7.7) or in cell culture obtained from flocks free from specified pathogens (2.7.7) or susceptible cell lines. The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10)

**Identification**

The vaccine, diluted if necessary and mixed with a monospecific chicken anaemia virus antiserum, no longer infects susceptible cell culture derived from SPF eggs (2.7.7) or egg from SPF flock (2.7.7) in to which it is inoculated.

**Tests**

Moisture (2.3.43): Not more than 3.0 per cent.

Mycoplasmas (2.7.9) Complies with the test for Mycoplasmas.

Safety. Use not fewer than 10 SPF chickens (2.7.7 table 3) or healthy susceptible chickens, not older than the minimum age recommended for vaccination (2.7.7). Administer by a recommended route to each chickens 10 doses of the vaccine. Observe the chickens 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 vaccine. The vaccine complies with the test if no chicken shows notable clinical signs of disease or dies from causes attributable to the vaccine.

Virus titer. Titrate the vaccine virus by inoculating into suitable cell lines or eggs from SPF flocks (2.7.7). One dose vaccine contains not less than $10^{3.0}$ TCID$_{50}$/EID$_{50}$ per dose.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out potency test for each of the routes of administration stated on the label. Vaccinate ten, 21 to 28 day old SPF chickens (2.7.7 table 3) or healthy susceptible chickens with one dose of vaccine. Keep 10 unvaccinated birds of the same age group as controls. Two to three weeks post vaccination challenge both the groups by intramuscular route with $10^2$ CID$_{50}$ CAV virus. Observe the birds for 14 days. Bleed individual birds for haematocrit value, thymus atrophy and bone marrow tissue discoloration.

The vaccine 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 and/or macroscopic lesions of the bone marrow and thymus.

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 stated on the label.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.
Labelling. The label/Insert states (1) strain of virus used; (2) dose of Vaccine.
Infectious Coryza Vaccine

Infectious coryza vaccine is a suspension of inactivated culture of suitable strains of 1 or more serotype/s or preferably locally prevalent strain/s of *Avibacterium (Haemophilus) paragallinarum* in a suitable medium.

**Production**

The seed material is inoculated in a suitable medium. If the vaccine contains more than 1 strain of bacterium, the different strains are grown and harvested separately. The bacterial harvests are inactivated with a suitable agent. The vaccine may contain suitable adjuvant.

**Identification**

Protects susceptible chicken against infection with *Avibacterium paragallinarium*.

**Tests**

**Sterility** (2.2.11). Complies with the test for sterility.

**Safety.** Inject double dose of vaccine sub-cutaneously into each of 10 SPF chickens (2.7.7 table 3) or healthy susceptible chickens at the minimum age group at which vaccine is intended. Observe these birds for 7 days; no bird shows untoward reactions other than slight transient local swelling.

**Potency.** Inject subcutaneously each of 10 SPF chickens (2.7.7 table 3) or healthy susceptible chickens of the minimum age group at which vaccine is used, with minimum dose stated on the label. Repeat the vaccination after 2 to 4 weeks. Use 10 healthy chickens of same age group and of same stock as controls. Two to three weeks later, challenge vaccinated and control chickens by instillation with 0.2 ml of 18-hour broth culture of homologous strain of *H.A. paragallinarium* diluted suitably so as to contain $1 \times 10^6$ colony forming units by infra-orbital sinus instillation. Observe the chickens for 7 days for unilateral eye swelling, nasal discharge. There should be not less than 70% protection of vaccinated birds. The test is not valid unless 70 per cent of control chickens exhibit typical symptoms of eye swelling and nasal discharge typical of infectious coryza.

**Storage.** When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date of potency testing.

**Labelling.** The label/Insert states (1) strains used for preparation; (2) route of administration.
Marek's Disease Vaccine, Live

Marek’s disease, Freeze dried / cell associatated vaccine (live) is a preparation of a suitable strain or strains of Marek’s Disease Virus (Avian Herpes Virus) or combinations their of.

Production

Preparation of the Vaccine

The vaccine virus is grown in cell cultures obtained from SPF (2.7.7) eggs. If the vaccine contains more than one type of virus, the different types are grown separately. The vaccine may be freeze-dried or stored in liquid nitrogen.

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Substrate for virus propagation

Cell culture derived from SPF eggs (2.7.7) obtained from SPF hens (2.7.7) eggs.

Identification

A. The vaccine on inoculation in susceptible cell cultures derived from SPF embryos causes cytopathic effects typical of Marek’s Disease virus.

OR

B. When mixed with a specific avian herpes virus antiserum the vaccine loses its capability to produce cytopathic effects or Plaques in susceptible cell cultures derived from SPF embryos.

Tests

Moisture (2.3.43). Not more than 3.0 per cent w/w (For Freeze dried vaccine only).

Mycoplasmas (2.7.9). Vaccine complies with the test for mycoplasmas.

Safety. Use ten one-day-old SPF chickens (2.7.7 table 3) or healthy susceptible chickens. Administer by recommended route and method to each chicken or chicken embryo 10 doses of the vaccine. Observe the chicken for 21 days. No chicken shows persistent clinical signs, dies or, at autopsy, shows macroscopic lesions from causes attributable to the vaccine. If during the observation period more than two chickens die from causes not attributable to the vaccine, repeat the test.

Sterility (2.2.11). Complies with the tests for sterility.

Virus titre. Vaccine containing one type of virus: Titerate the vaccine virus by inoculation into suitable cell culture derived from SPF eggs (2.7.7). If the virus titer is determined in plaque forming units (PFU), only primary plaques are taken into consideration. The vaccine complies with the test if one dose contains not less than 10^3 PFU per dose.

Vaccine containing more than one type of virus: For vaccine containing more than one type of virus, titrate each virus by inoculation into suitable cell culture derived from SPF eggs (2.7.7)s, reading the results by immunostaining using antibodies. Vaccine complies with the test if one dose contains for each vaccine virus not less than 10^3 PFU of virus per dose.

Potency. Carry out a separate potency test for each of the routes of administration stated on the label. For each of the stated routes, use not less than thirty susceptible one-day-old SPF chickens (2.7.7 table 3) or healthy susceptible chickens.
Administer each chicken a volume of the vaccine containing a quantity of the virus equivalent to the minimum titre stated on the label. Use thirty chickens of the same flock and age as controls. After 9 days, challenge each chicken by a suitable route with a suitable quantity of virulent Marek's disease virus. Observe the birds for 10 weeks. Record the deaths and kill the survivors to carry out autopsies on both dead and sacrificed chicken for specific macroscopic lesions of Marek's disease. For each of the stated routes of administration, the total number of vaccinated birds that show specific macroscopic lesions is reduced by not less than 80% as compared with the control birds and the challenge virus produces specific macroscopic lesions in not less than 70% of the control birds.

If the potency test has been performed with satisfactory results on representative batch of the vaccine from the same seed lot, it may be omitted as a routine control test during production of other batches of the vaccine prepared from the same seed lot.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

Labelling. The label states (1) the minimum virus titre; (2) dose of Vaccine.

The frozen vaccine has to be dispensed in glass ampoules suitable for liquid nitrogen storage and if the above information cannot be printed on the small size ampoule, the product should be accompanied by suitable insert which clarifies the prescribed contents of the labels.
Newcastle Disease Vaccine, Inactivated / Ranikhet Disease Vaccine, Inactivated

Ranikhet Disease Vaccine, Inactivated consists of an emulsion or a suspension of a suitable strain of Newcastle disease virus (avian paramyxovirus 1) that has been inactivated in such a manner that immunogenic activity is retained.

Production

Substrate for Propagation

The vaccine virus is grown either in embryonated hens’ eggs or in cell culture derived from SPF eggs (2.7.7)s or suitable cell line.

The master seed lot complies with the tests for extraneous agents as prescribed in the General monograph for Veterinary Vaccines (2.7.10).

Inactivation.

Inject quantity of inactivated virus equivalent to 10 doses of vaccine into the allantoic cavity of each of 10 embryonated 9 to 11 days old SPF eggs (2.7.7), and incubate. Observe for 6 days and pool separately the allantoic fluid from eggs containing live embryos and that from eggs containing dead embryos, excluding those dying within 24 hours of the injection. Examine embryos that die after 24 hours of injection for the presence of Newcastle disease virus. Test the allantoic fluid from each egg for the presence of haemagglutinins using chicken erythrocytes.

Inject into the allantoic cavity of each of 10 SPF eggs (2.7.7), 9 to 11 days old, 0.2 ml of the pooled allantoic fluid from the live embryos and, into each of 10 similar eggs, 0.2 ml of the pooled fluid from the dead embryos and incubate for 5 to 6 days. Test the allantoic fluid from each egg for the presence of haemagglutinins using chicken erythrocytes.

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.

Identification

When injected into susceptible healthy chicken, the vaccine stimulates the production of specific antibodies against Newcastle disease virus.

Tests

Safety. Inject ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens of the age stated on the label with twice the dose and by the route stated on the label. Observe the birds for 21 days. No abnormal local or systemic reactions are observed.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Either test A or test B may be carried out.

Test A. Inject intramuscularly each of ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens, between 3 - 4 weeks old, with a volume of the vaccine equivalent to one-fiftieth of a dose. Use ten chickens of the same stock and age group as controls. After 21 days, collect serum samples from each of the vaccinated and unvaccinated chicken. Perform haemagglutination inhibition test using the method.
described below. Use the positive control serum calibrated against a Standard preparation of anti-Newcastle disease serum. The vaccine passes the test if a mean HI titer of the vaccinated group is equal to or greater than 1:16 and that of the unvaccinated controls is equal to or less than 1:4.

If the HI titers are not satisfactory, carry out the test B.

**Standard preparation**

The Standard preparation is the 1st International reference preparation, established in 1966, consisting of freeze-dried chicken serum (supplied in ampoules containing 320 Units), or another suitable preparation, the potency of which has been determined in relation to the International reference preparation.

**Suggested method of haemagglutination inhibition test.** Inactivate the serum samples by heating at 56° for 30 minutes. Add 0.05 ml of saline solution to all the wells in a microtitre plate and 0.05 ml of the test sera to the first row of wells. Prepare two-fold dilutions of the serum samples across the plate. Add 0.05 ml of a suspension of Newcastle disease virus containing 4 haemagglutinating units of inactivated Newcastle disease virus. Incubate the plate at 4° for one hour. Add 0.05 ml of a 1 per cent suspension of erythrocytes collected from chicken, between 3 - 4 weeks old, susceptible to Newcastle disease.

Incubate the plate at 4° for one hour. It must be ensured that negative and positive control sera are included in the test. The positive control serum must show a titre of 300 to 400 Units determined by calibration against the Standard reference Preparation.

**Test B.** Inject intramuscularly each of three groups of twenty SPF chickens (2.7.7 table 3) or healthy susceptible chickens, between 3 - 4 weeks old, with five fold dilution of vaccine. Use minimum three dilutions. Allocate a different volume to each vaccination group. Vaccinate each chicken by the intramuscular route with the volume of vaccine allocated to its group. Maintain not less than 10 chickens as controls. Challenge each chicken after 21 days by the intramuscular route with 10⁶ chick LD₅₀ of the virulent strain of avian Paramyxovirus 1. Observe the chickens at least daily for 7 days after challenge. At the end of the observation period, calculate the PD₅₀ by standard statistical methods from the number of chickens that survive in each vaccinated group without showing any signs of Newcastle disease during the 7 days. The vaccine complies with the test if the smallest dose stated on the label corresponds to not less than 50 PD₅₀ and the lower confidence limit is not less than 35 PD₅₀ per dose. If the lower confidence limit is less than 35 PD₅₀ per dose, repeat the test; the vaccine must be shown to contain not less than 50 PD₅₀ in the repeat test. The test is not valid unless all the control birds die within 6 days of challenge.

**Storage.** When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

**Labelling.** The label/Insert states (1) strain of virus used; (2) route of administration.
Ranikhet Disease Vaccine, Live (Lentogenic Strain)

Newcastle Disease Vaccine, Live (Lentogenic strain)

Ranikhet Disease Vaccine Live (Lentogenic Strain) is a preparation of a suitable strain of Newcastle disease/Ranikhet disease virus (avian paramyxovirus 1). This monograph applies to vaccines intended for administration to chickens and/or other avian species for active immunization.

Production

Substrate for Propagation

The vaccine virus is grown in embryonated SPF eggs (2.7.7) or in cell cultures derived from SPF flocks (2.7.7).

The master seed lot complies with the tests for extraneous agents as described prescribed in the General monograph for Veterinary Vaccines (2.7.10).

Identification

The vaccine, diluted if necessary and mixed with a monospecific Newcastle disease virus antiserum, no longer provokes haemagglutination of chicken red blood cells or infects embryonated hens’ eggs from SPF flock or susceptible cell culture derived from SPF eggs (2.7.7)s into which it is inoculated.

Tests

Moisture (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). Vaccine complies with the test for mycoplasmas.

Safety. For vaccines recommended for use in healthy susceptible chickens, use not less than 10 SPF chickens (2.7.7 table 3) or healthy susceptible chickens demonstrated to be free from antibodies to Newcastle disease virus and of the youngest age recommended for vaccination. For vaccines recommended for use only in avian species other than the chicken, use not fewer than 10 birds of the species likely to be most sensitive to Newcastle disease, which do not have antibodies against Newcastle disease virus and of the minimum age recommended for vaccination. Administer to each bird by eye-drop, or parenterally if only parenteral administration is recommended, 10 doses of the vaccine in a volume suitable for the test. Observe the birds at least daily for 21 days. The test is not valid if more than 20 per cent of the birds show abnormal clinical signs or die from causes not attributable to the vaccine. The vaccine complies with the test if no bird shows notable clinical signs of disease or dies from causes attributable to the vaccine.

Virus titre. Not less than 10^6 TCID₅₀/EID₅₀ of the virus per dose, determining the titre in suitable cell culture derived from SPF eggs (2.7.7) or by inoculation into the allantoic cavity of SPF embryonated eggs, 9 to 11 days old.

Sterility (2.2.11). Complies with the test for sterility.

Potency (2.2.11). Carry out a potency test for each of the routes of administration stated on the label. For each of the stated routes, use at least ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens and of the minimum age recommended for vaccination.

Administer each chicken with a volume of the reconstituted vaccine containing a quantity of the virus equivalent to the minimum titre stated on the label. Use ten chickens of the same flock and age as controls. After 14 to 21 days, challenge each chicken by intramuscular injection with 10^5 LD₅₀, of a virulent strain of Newcastle disease virus. Observe the chickens for 14 days. The vaccine complies with the test if not
more than two of the vaccinated chickens die or show signs of disease. The test is valid only if all the control birds die within 6 days of inoculation of the virulent challenge strain.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

Labelling. The label/insert states (1) strain of virus used; (2) dose of Vaccine.
Ranikhet Disease Vaccine, Live (Mesogenic Strain)

Ranikhet Disease Vaccine, Live (Mesogenic Strain) is a preparation of a suitable strain of Newcastle disease virus (naturally modified avian Paramyxovirus 1). This monograph applies to vaccines intended for administration to chickens for active immunization.

Production

Substrate for Propagation

The vaccine virus is grown in embryonated SPF eggs (2.7.7) or in cell cultures derived from SPF flocks (2.7.7) or susceptible cell lines. The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Identification

The vaccine, diluted if necessary and mixed with a monospecific Newcastle disease virus antiserum, no longer provokes haemagglutination of chicken red blood cells or infects embryonated hens’ eggs from SPF flock (2.7.7) or susceptible cell culture derived from SPF eggs (2.7.7)s into which it is inoculated.

Tests

Moisture (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). Vaccine complies with the test for mycoplasmas

Safety. Administer fifteen SPF chickens (2.7.7 table 3) or healthy susceptible chickens, 8 to 9 weeks old, with a minimum 10 doses and by the route stated on the label. Observe the chickens for 21 days. None of them Not more than 2 chicken shows abnormal clinical signs or dies due to causes attributable to the vaccine. If more than two chickens die during the period of observation due to causes other than those attributable to the vaccine, repeat the test.

Virus titre. Not less than $10^5$ TCID50/EID50 of the virus per dose, determining the titre in suitable cell culture derived from SPF eggs (2.7.7) or by inoculation into the allantoic cavity of SPF embryonated eggs (2.7.7), between 9-11 days old.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out potency test for each of the routes of administration stated on the label. For each of the stated routes, use not less than ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens and of the minimum age recommended for vaccination. Administer each chicken with a volume of the reconstituted vaccine containing a quantity of the virus equivalent to the minimum titer stated on the label. Use ten chickens of the same flock and age as controls. After 14 to 21 days, challenge each chicken by intramuscular injection with $10^5$ LD50 of a virulent strain of Newcastle disease virus. Observe the birds for 14 days. The vaccine complies with the test if not more than two of the vaccinated chickens die or show signs of disease. The test is valid only if all the control chickens die within 6 days of inoculation of the virulent challenge strain.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.
Labelling. The label/Insert states (1) strain of virus used; (2) dose of Vaccine.
Reo Virus Vaccine, Inactivated

Reo virus vaccine, Inactivated consists of an emulsion or a suspension of a suitable strain/s of Reo virus which has been inactivated in such a manner that immunogenic activity is retained. The vaccine may contain one or more strains and a suitable adjuvant.

Production

Substrate for propagation

The virus is propagated in fertilized eggs obtained from healthy flock or in suitable cell culture derived from SPF flocks (2.7.7) or susceptible cell line.

The master seed lot complies with the tests for extraneous agents as described prescribed in the General monograph for Veterinary Vaccines (2.7.10)

Inactivation

An amplification test for residual live Infectious Avian Reo Virus is carried out on each batch of antigen immediately after inactivation and the test is carried out in fertilized SPF hen eggs or in suitable cell culture derived from SPF eggs (2.7.7). The quantity of inactivated virus used in the test is equivalent to not less than ten doses of the vaccine. No live virus is detected.

Test for Inactivation

In Cell culture derived from SPF eggs (2.7.7). Inoculate 10 doses of vaccine into suitable cell culture derived from SPF eggs (2.7.7) derived from SPF eggs (2.7.7). Incubate at 36±1°C for 7 days. Make a passage on another set of cell culture derived from SPF eggs (2.7.7) and incubate at 36±1°C for 7 days. None of the cultures shows signs of infection i.e. CPE.

Embryonated eggs. Inject quantity of inactivated virus equivalent to 10 doses of vaccine into the allantoic cavity of the SPF embryonated hen eggs, between 9-11 days old, and incubate at 36±1°C. Observe for 6 days and pool separately the allantoic fluid from eggs containing live embryos, and that from eggs containing dead embryos, excluding those dying from non-specific causes within the first 24 hours after inoculation. Inject into the allantoic cavity of each of the SPF embryonated hen eggs derived from SPF eggs (2.7.7) and incubate at 36±1°C for 6 days. Examine each embryo for lesions of Reo virus. The vaccine complies with the test if there is no evidence of lesions of Reo virus. The test is valid only if not more than 20 per cent of the embryos die at either stage of the test. If more than 20 per cent of the embryos die at either one of the stages of the test, repeat that stage. In any repeat test, not more than 20 per cent of the embryos die from non-specific causes. Antibiotics may be used to control extraneous bacterial infection.

Identification

In susceptible chickens, the vaccine stimulates the production of specific antibodies against each of the virus serotypes in the vaccine detected by virus neutralization.

Tests

Safety. Inject each of ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens, 14 to 28 days old with twice the minimum vaccinating dose and by one of the routes stated on the label. Observe the chickens for 14 days. No abnormal local or systemic reaction should be seen.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject each of twenty SPF chickens (2.7.7 table 3) or healthy susceptible chickens, 3 to 4 weeks old, with the minimum dose and by the route stated on the label. Use ten chickens of the same flock and
age as controls. After 21 days, collect serum samples from each bird including the ten-control chickens and perform quantitative agar gel precipitation test or serum neutralization test on each serum sample. The mean antibody titer of sera in vaccinated group shall be 1:8 by Agar gel diffusion test and 10000 units per ml by serum neutralization test and there should be no specific antibodies in the sera of control chicken.

**Storage.** When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

**Labelling.** The label/insert states (1) strains used for preparation; (2) Route of administration.
Reo Virus Vaccine, Live

Reo vaccine live is a preparation of a suitable strain(s) of Reo virus known to be safe and immunogenic. This monograph applies to vaccines intended for administration of chickens for protection against Malabsorption Syndrome and/or proventriculitis and/or Tenosynovitis in birds.

Production

Substrate for Propagation

The vaccine virus is grown in embryonated SPF hens’ eggs or in cell cultures derived from SPF flocks (2.7.7) or suitable cell line.

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines.

Identification

When mixed with monospecific Reo virus antiserum, the vaccine no longer induces cytopathic effect in susceptible cell culture derived from SPF eggs (2.7.7) or carry out immunostaining test in cell culture derived from SPF eggs (2.7.7)s to identify the vaccine virus.

Tests

Moisture (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). Vaccine complies with the test for mycoplasmas.

Safety. Final container samples of completed product from each serial shall be tested as follows:

(A) For vaccines intended for use in very young chickens, each of 10, one day old SPF chickens (2.7.7 table 3) or healthy tenosynovitis/malabsorption/proventriculitis susceptible chickens shall be vaccinated with the equivalent of 10 doses by one method recommended on the label.

(B) For vaccines intended for use in older chickens, each of ten, 4-week-old or older SPF chickens (2.7.7 table 3) or healthy tenosynovitis susceptible chickens shall be vaccinated with the equivalent of 10 doses by one method recommended on the label.

The vaccinates shall be observed each day for 21 days. If unfavorable reactions occur which are attributable to the product, the serial is unsatisfactory. If unfavorable reactions occur in more than two vaccinates which are not attributable to the product, the test is inconclusive and may be repeated. If the test is not repeated, the serial is unsatisfactory.

Virus Titre. Titrate the vaccine in cell cultures derived from SPF embryos or in SPF eggs (2.7.7). One dose of the vaccine contains not less than $10^3$ TCID$_{50}$ / EID$_{50}$ per dose.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Reo susceptible healthy chickens of same age and from the same source shall be used as test birds. Vaccine intended for use in very young chickens shall be administered to chickens of the youngest age for which vaccine is recommended. Vaccines intended for use in older chickens shall be administered to 4 weeks or older birds. Ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens vaccinates shall be used for each method of administration. One dose will injected to vaccinates. Ten chicks shall be held as unvaccinated controls.
Potency test of each age group shall be conducted separately. Twenty one days post vaccination each vaccinates and control shall be challenged by injecting virulent virus into one foot pad. The vaccinates & controls shall be observed for 14 days post challenge. If at least 90% of the controls do not develop swelling and discoloration in the phalangeal joint area of injected foot pad typical of infection of Reo virus, the test is inconclusive and may be repeated. If at least 18 out of 20 vaccinates do not remain free of these signs, disregarding transient swelling which subsides within 5 days post challenge, the serial is unsatisfactory. The serial is satisfactory when it gives 90% protection to vaccinated group and 90% controls develop positive Reo virus lesions on challenge.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

**Storage.** When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

**Labelling.** The label/Insert states (1) strain of virus used (2) dose of vaccine.
Salmonella Vaccine, Inactivated

Salmonella vaccine inactivated is a preparation of 1 or more suitable strains of 1 or more serovars of Salmonella organism, inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunization of chickens against infection/s of salmonella in chickens and reducing Salmonella colonization and fecal excretion in chickens.

Production
The seed material is inoculated in a suitable medium. If the vaccine contains more than 1 strains of bacterium, the different strains are grown and harvested separately. During production parameters such as growth rate, purity and identity is verified on harvests using suitable culture. The bacterial harvests are inactivated with suitable agent. The vaccine may contain suitable adjuvant.

Identification
Vaccine stimulates production of strain specific antibodies against Salmonella organisms in susceptible birds.

Tests
Safety. Administer double dose of vaccine subcutaneously into each of ten SPF chickens (2.7.7 table 3) or healthy susceptible chickens of minimum age recommended for vaccination. Observe the birds at least for 21 days. The test is not valid if more than 20 per cent of the chickens show abnormal 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.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out separate potency test for each strain of salmonella organism incorporated in the vaccine preparation. Use not less than 10 SPF chickens (2.7.7 table 3) or healthy susceptible chickens of the minimum age recommended for vaccination. Administer 1 dose of vaccine by a recommended route. Maintain 10 chickens as unvaccinated controls from the same source and flock used for vaccination for each strain used in vaccine. Repeat the vaccination with the same dose and route after 21 days to vaccinated birds. Challenge both the groups, 4-2 weeks after last administration of vaccine, by oral administration to each chicken a sufficient quantity of a homologous strains of Salmonella organisms that is able to colonize chickens. Observe the birds’ daily for 14 days. Collect fecal samples on 14th day for detection of presence of Salmonella organisms by direct plating. The vaccine complies with the test, if the numbers of Salmonella organisms in fresh fecal samples after challenge is significantly lower in vaccinated birds than in unvaccinated controls.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined

Labelling. The label states (1) strains used for preparation; (2) route of administration.
Salmonella Pullorum Antigen

Salmonella Pullorum Antigen is a suspension of a pure smooth culture of representative strains of *Salmonella pullorum* which are of known antigenic composition, high agglutinability, but are not sensitive to negative and non-specific serum. Each intermediate lot shall be tested for purity, density, and preservative.

**Purity.** Intermediate lot sample should be free from extraneous organisms as determined by microscopic examination and gram staining.

**Density.** The bacterial density shall be 80±15 times Mac-Farland Number 01 for stained antigen and 50±10 times Mac-Farland Number 01 for tube antigen.

**Preservative**
Formalin contents of the intermediate lot of colored antigen shall be 1.0±0.2 per cent. Phenol contents of plain antigen shall be 0.55±0.5 per cent.

A batch of finished product should be tested for Identification, homogeneity and Hydrogen Ion Concentration. The batch of finished product found unsatisfactory for any prescribed test shall not be released.

**Tests**

**Identification**
Gives specific agglutination when mixed with the serum of birds infected with S. pullorum or S. gallinarum but fails to react with serum from healthy birds.

**Homogeneity.** Antigen shall show no evidence of auto agglutination or unusual appearance such as presence of flakes.

**pH.** Hydrogen Ion concentration shall be determined with a pH meter which has been standardize with pH 4.0 buffer just prior to use. The pH of stained antigen shall be 4.6±0.4. No pH level is specified for pullorum tube antigen but after dilution, as recommended for use it shall have a pH of 8.2 to 8.5.

**Storage.** When stored under the prescribed conditions, the antigen may be expected to retain its potency for not less than 1 year from the date the potency was determined.

**Labelling.** The label states (1) strains used for preparation; (2) dose of Test.
Avian Mycoplasma Antigen

Mycoplasma antigen shall be prepared either from Mycoplasma gallisepticum or Mycoplasma synoviae, grown in broth cultures that are inactivated and standardized. Plate antigen shall be stained with an acceptable dye. Each intermediate antigen lot shall be tested for purity, density, and preservative.

**Purity.** Intermediate antigen lot sample should be free from extraneous organisms as determined by microscopic examination and gram staining.

**Density.** A 2.5 ml of sample of intermediate lot shall be diluted with 2.5ml of buffer solution, formulated in the same manner as the vehicle of the antigen being tested in a modified Hopkins tube and then sedimented by centrifugation. If the packed cell volume of the sample is not 1.2± 0.4 per cent, the intermediate antigen lot is unsatisfactory.

**Preservative**
Phenol contents of antigen lot shall be 0.25±0.05 per cent.

A batch of finished product should be tested for Identification, homogeneity and Hydrogen Ion Concentration. The batch of finished product found unsatisfactory for any prescribed test shall not be released.

**Tests**

**Identification**
Gives specific agglutination when mixed with the serum of birds infected with M. gallisepticum or M. synoviae but fails to react with serum from healthy birds.

**Homogeneity**
Antigen shall show no evidence of auto agglutination or unusual appearance such as presence of large visible particles.

**Hydrogen Ion Concentration**
Hydrogen Ion concentration shall be determined with a pH meter which has been standardize with buffer just prior to use. The pH of mycoplasma gallisepticum antigen shall be 6.0±0.2. The pH of mycoplasma synoviae antigen shall be 7.0±0.2.

**Storage.** When stored under the prescribed conditions, the antigen may be expected to retain its potency for not less than 1 year from the date the potency was determined.

**Labelling.** The label states (1) strains used for preparation; (2) Dose of Test.
Sterile Diluent for Live Vaccines

Sterile diluents are required for reconstitution of freeze dried and frozen vaccines. The sterile diluents may be a special liquid solution.

Each diluent batch shall be given a number which shall be used in records, test reports and final containers.

Tests

Sterility (2.2.11). Complies with the test for sterility.

Viral Stability
Each batch should be tested for viral stability by holding two hours after reconstitution of vaccine at recommended temperature.

Hydrogen Ion Concentration
Hydrogen Ion concentration shall be determined with a pH meter which has been standardized with buffer.

Clarity
Each batch should be free from visible particulate matter.