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CONTENTS

AVIAN VACCINES

SWINE VACCINES

RUMINANT VACCINES

SMALL ANIMAL VACCINES

APPENDIX

ASEAN STANDARD REQUIREMENTS FOR AVIAN ENCEPHALOMYELITIS VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as Appendix 1.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples.

2. PURITY TEST

Seedlot or bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods that appear as Appendix 3.

3. SAFETY TEST

Final container samples should be tested by the following:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 10 doses of the vaccine by the recommended routes and observed for a minimum of 21 days. No clinical signs of Avian Encephalomyelitis (AE) should be observed in any of the chickens.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of the vaccine by the recommended routes. At least 21 days post-vaccination, the vaccinates together with 10 unvaccinated controls are challenged with virulent AE virus by the intracerebral or intramuscular route and observed for a minimum of 21 days. At least 80% of the vaccinates should survive and show no clinical signs of the disease and at least 80% of the controls should die or show clinical signs of AE.

5. VIRUS CONTENT

The vaccine should have a virus titre of not less than $10^{2.5}$ EID_{50} per dose when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as Appendix 4.

ASEAN STANDARD REQUIREMENTS FOR AVIAN ENCEPHALOMYELITIS VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as Appendix 1 or healthy flocks.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, fungi, Salmonella, and Mycoplasma by the methods that appear as Appendix 2**.** However, tests for Salmonella and Mycoplasma may be carried out on bulk samples. The test for Mycoplasma may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods which appear as Appendix 3. This test may be omitted if it can be demonstrated that the inactivating agent inactivates avian leucosis viruses.

3. INACTIVATION TEST

At least 10 embryonated chickens eggs susceptible to avian encephalomyelitis (AE) virus are each inoculated with 0.2 ml of the inactivated product by the yolk sac route. The eggs are incubated for a minimum of 14 days. Brain samples are harvested from the embryos for a

second passage. The inoculated eggs are examined after at least 14 days for signs of AE lesions. There should be no evidence of AE virus.

4. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with at least 2 doses of vaccine by the recommended route and observed for a minimum of 14 days. No abnormal local or systemic reaction attributable to the vaccine should occur in any of the chickens.

5. POTENCY TEST

Bulk or final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with with 1 dose of the vaccine by the recommended route. At least 21 days post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged with virulent AE virus by the intracerebral route and observed for 14 days. At least 80% of the vaccinates should survive and show no clinical signs of disease and at least 80% of the controls should develop clinical signs of the disease or die.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as Appendix 4.

ASEAN STANDARD REQUIREMENTS FOR DUCK PLAGUE VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs or cell culture in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs or cell cultures used throughout the production of the vaccine must be derived from specific-pathogen-free (SPF) flocks complying with tests that appear as Appendix 1.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples.

2. PURITY TEST

Seedlot or bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods that appear as *Appendix 3*.

3. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible ducks of the minimum age for which the vaccine is intended, are each inoculated with 10 doses of the vaccine by the recommended routes. The ducks are observed clinically for 2 weeks. The

vaccine is considered satisfactory if all of the ducks do not show any adverse systemic and local reactions.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows:

At least 20 susceptible ducks of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of the vaccine by the recommended route. At least 2 weeks post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged intramuscularly with 100 LD_{50} of a virulent Duck Plaque virus and observed for a minimum of 2 weeks post-challenge. At least 90% of the vaccinated ducks should remain healthy and show no clinical signs of the disease and at least 90% of the controls should die of Duck Plaque.

5. VIRUS CONTENT

The vaccine should have a virus titre of not less than $10^{3.0}$ EID_{50} or $TCID_{50}$/dose when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as Appendix 4.

ASEAN STANDARD REQUIREMENTS FOR EGG DROP SYNDROME 76 VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The master and working seed viruses are produced in embryonated duck eggs derived from healthy flocks in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Suitable cell culture or embryonated eggs used throughout production of the vaccine must be derived from healthy hen or duck flocks.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, fungi, Salmonella, and Mycoplasma by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples. The test for Mycoplasma may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods that appear as Appendix 3. This test may be omitted if it can be demonstrated that the inactivating agent inactivates avian leucosis viruses.

3. INACTIVATION TEST

At least 10 embryonated duck eggs susceptible to Egg Drop Syndrome 76 (EDS) virus are each inoculated with 0.2 ml of the inactivated product by the allantoic sac route. The eggs are incubated for a minimum of 3-7 days. One subculture is carried out. There should be no evidence of EDS virus. The

test may be carried out in chicken embryo liver or duck embryo fibroblast cell cultures.

4. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with at least 2 dose of the vaccine by the recommended routes. The chickens are observed clinically for 14 days. No abnormal local or systemic reactions attributable to the vaccine should occur in any of the chickens.

5. POTENCY TEST

Bulk or final container samples should be tested by one of the following methods:

a) At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 1/50 dose of vaccine by the recommended routes. At least 5 chickens are kept as unvaccinated controls. Three to 4 weeks post-vaccination, all chickens are serologically tested. At least 50% of the vaccinates should have haemagglutination inhibition (HI) antibody titres of at least 1: 16. The controls should remain negative.

b) At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of vaccine by the recommended routes. At least 5 chickens are kept as unvaccinated controls. Three to 4 weeks post-vaccination, all chickens are serologically tested. The mean HI titre of the vaccinated group should be greater than 1: 128.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR FOWL POX VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as Appendix 1.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples.

2. PURITY TEST

Seedlot or bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods that appear as *Appendix 3*.

3. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 10 doses of the vaccine by the recommended routes. The chickens are observed clinically for 3 weeks. The vaccine is considered satisfactory if all of the chickens do not show any adverse clinical signs of the disease.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of vaccine by the recommended routes. At least 2 weeks post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged by scarification with virulent Fowl Pox virus and observed for 10 days. At least 90% of the vaccinates should not develop any lesions of Fowl Pox and 90% of the controls should show lesions.

5. VIRUS CONTENT

The vaccine should have a virus titre of not less than $10^{2.0}$ log EID_{50} per dose or 10 Minimum Protective Dose per dose (whichever is higher) when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master and working seed culture should be *Pasteurella multocida* of known serotypes virulent for the intended avian species and produced in a seed lot system. The seed bacteria must satisfy purity tests before they are used for bacterin production. They must be dispensed into separate containers and stored at lower than –18^{O}C.

2. PRODUCTION SUBSTRATE

The medium should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of extraneous bacteria and fungi by the methods that appear as *Appendix 2.*

2. PURITY TEST

Bulk production samples should be tested as follows:

Gram stained smears of live cultures are examined for morphological characteristics. Only *P. multocida* should be present.

3. INACTIVATION TEST

The bulk or final product is tested for inactivation by culturing in a medium known to support growth of *P. multocida*. No *P. multocida* or any other bacteria should be detected.

4. SAFETY TEST

Final container samples should be tested in the species intended as follows:

At least 10 susceptible fowls are each inoculated with 2 doses of bacterin by the recommended route and observed for 14 days. No abnormal local or systemic reaction attributable to the bacterin should occur in any of the fowls.

5. POTENCY TEST

Bulk or final container samples should be tested in the species intended as follows:

At least 20 susceptible fowls of the minimum age for which the bacterin is intended, are each vaccinated by the recommended route. At least 1 week after the vaccination, the vaccinated fowls together with at least 10 susceptible unvaccinated controls are each challenged intramuscularly with virulent *P. multocida* [local strain (s)] and observed for a minimum of 10 days. At least 80% of the vaccinated fowls should be protected and at least 80% of the controls should die.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master and working seed culture should be *Haemophilus paragallinarum* produced in a seed lot system. The seed bacteria must satisfy purity tests before they are used for bacterin production. The seed bacteria are lyophilized or kept in small vials and stored at -18^0C or lower.

2. PRODUCTION SUBSTRATE

The medium should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of extraneous bacteria and fungi by the methods that appear as *Appendix 2.*

2. PURITY TEST

Bulk production samples should be tested as follows:

Gram stained smears of live cultures are examined for morphological characteristics. Only *H. paragallinarum* should be present.

3. INACTIVATION TEST

The bulk or final product is tested for inactivation by culturing in a medium known to support growth of *H. paragallunarum* e.g. Blood agar cross-streaked with *Staphylococcus aureus*; brain heart infusion agar supplemented with horse serum and nicotinamide adenine dinucleotide (NAD) or reduced NAD (NADH); chicken meat infusion broth supplemented with chicken serum.

Incubation is carried out in enriched CO_2 atmosphere at 37°C for 24 to 48 hours. No *H. paragallinarum* or any other bacteria should be detected.

4. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with at least 2 doses of bacterin by the recommended route and observed for a minimum of 21 days. No abnormal local or systemic reaction attributable to the bacteria should occur in any of the chickens.

5. POTENCY TEST

Bulk or final container samples should be tested by one or more of the following methods :

a) At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each vaccinated as recommended. At least 2 weeks after the final vaccination, the chickens are divided into groups with at least 10 vaccinates and 10 unvaccinated controls per group for each serotype of *H. paragallinarum* contained in the bacterin. Each group is challenged with a virulent homologous serotype of *H. paragallinarum* and observed for 10 days. At least 70% of the vaccinates should remain free of clinical signs of Infectious Coryza and at least 70% of the controls should show clinical signs.

b) At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each vaccinated as recommended. At least 2 weeks after the final vaccination, the vaccinates and 10 unvaccinated controls are each serologically tested for each serotype of *H. paragallinarum* contained in the bacterin. The difference between the mean antibody titre of the vaccinates and the controls should be at least $10^{0.6}$.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR INFECTIOUS BRONCHITIS VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as Appendix 1.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples.

2. PURITY TEST

Seedlot or bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods that appear as *Appendix 3*.

3. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 10 doses of the vaccine by the recommended routes. The chickens are observed clinically for 3 weeks. The vaccine is considered satisfactory if at least 90% of the chickens do not show any adverse clinical signs of the disease.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows:

At least 20 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of vaccine by the recommended route. At least 21 days post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged with the homologous virulent IB virus by either the ocular or intratracheal route. At 4 to 7 days post-challenge, tracheal swabs or mucosal scrapings are obtained from the chickens and assayed individually for IB virus by inoculating into the allantoic sac of susceptible embryonated chicken eggs. Inoculated eggs are incubated for a minimum of 7 days. All embryos that die after 24 hours and those that survive are examined for lesions characteristic of IB infection. At least 80% of the vaccinates should be free of IB virus. The virus should be recovered from at least 80% of the controls.

5. VIRUS CONTENT

The vaccine should have a virus titre of not less than $10^{2.0}$ EID_{50} per dose or 10 Minimum Protective Dose per dose when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR INFECTIOUS BRONCHITIS VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as *Appendix 1* or healthy flocks.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, and fungi, Salmonella, and Mycoplasma by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples. The test for Mycoplasma may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods that appear as *Appendix 3*. This test may be omitted if it can be demonstrated that the inactivating agent inactivates avian leucosis viruses.

3. INACTIVATION TEST

At least 10 embryonated chickens eggs susceptible to infectious bronchitis (IB) virus are each inoculated with 0.2 ml of the inactivated product

by the allantoic sac route. The eggs are incubated for a minimum of 7 days. One subculture is carried out. There should be no evidence of IB virus.

4. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with at least 2 doses of vaccine by the recommended route and observed for a minimum of 21 days. No abnormal local or systemic reaction attributable to the vaccine should occur in any of the chickens.

5. POTENCY TEST

Bulk or final container samples should be tested as follows:

At least 20 susceptible chickens of the minimum age for which the vaccine is intended, are each vaccinated with 1 dose of vaccine by the recommended route. At least 2 weeks post-vaccination, the vaccinates together with 20 unvaccinated controls are serologically tested. The geometric mean haemagglutination inhibition titre of the vaccinates should be greater than 1:20 and all controls should remain negative.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR INFECTIOUS BURSAL DISEASE VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as Appendix 1.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples.

2. PURITY TEST

Seedlot or bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods that appear as Appendix 3.

3. SAFETY TEST

Master seed or final container samples should be tested as follows:

At least 15 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with either 1 or 10 dose of vaccine by the recommended route. At the end of the 3 weeks observation period, all chickens are killed and examined histologically (if vaccinated with 1 dose) or grossly (if vaccinated with 10 doses). No clinical signs of lesions of IBD should be detected in any of the chickens.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows:

At least 20 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of vaccine by the recommended route. At 10 to 14 days post-vaccination, the vaccinates together with 5 unvaccinated controls are challenged with virulent IBD virus by the ocular route. The chickens are necropsied and examined grossly at 3 to 5 days post-challenge or histologically at 10 days post-challenge. At least 80% of the vaccinates should not have lesions of IBD and at least 80% of the controls should have lesions.

5. VIRUS CONTENT

The vaccine should have a virus titre of not less than 50 Minimum Protective Dose per dose when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR INFECTIOUS BURSAL DISEASE VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as *Appendix 1* or healthy flocks.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria and fungi, Salmonella, and Mycoplasma by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples. The test for Mycoplasma may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods that appear as *Appendix 3*. This test may be omitted if it can be demonstrated that the inactivating agent inactivates avian leucosis viruses.

3. INACTIVATION TEST

At least 10 embryonated chickens eggs susceptible to infectious bursal disease (IBD) virus are each inoculated with 0.2 ml of the inactivated product by the chorioallantoic membrane, yolk sac or allantoic sac routes. The eggs

are incubated for a minimum of 7 days. One subculture is carried out. There should be no evidence of IBD virus. Cell culture adapted vaccines may be tested using susceptible cell cultures.

4. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with at least 2 doses of vaccine by the recommended route and observed for a minimum of 21 days. No abnormal local or systemic reaction attributable to the vaccine should occur in any of the chickens.

5. POTENCY TEST

Bulk or final container samples should be tested by one or more of the following methods:

a) At least 20 susceptible, 2 to 4 weeks-old chickens, are each vaccinated with 1 dose of vaccine by the recommended route. At 21 to 28 days post-vaccination, the vaccinates together with 5 unvaccinated controls are each challenged with virulent IBD virus. The chickens are necropsied and examined grossly at 3 to 5 days post-challenge or histologically at 10 days post-challenge. At least 80% of the vaccinates should not have lesions of IBD and at least 80% of the controls should show lesions.

b) At least 20 susceptible, 2 to 4 week-old chickens are each vaccinated with 1 dose of vaccine by the recommended route. At 3 to 4 weeks post-vaccination, the vaccinates together with 5 unvaccinated controls are each serologically tested. The mean AGP antibody titre of the vaccinates should be at least 1:32 and all controls should remain negative.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR INFECTIOUS LARYNGOTRACHEITIS VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as Appendix 1.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as *Appendix 2*. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples.

2. PURITY TEST

Seedlot or bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods which appear as *Appendix 3*.

3. SAFETY TEST

Final container samples should be tested as follows:

At least 20 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 10 doses of the vaccine by the intra-tracheal route. The chickens are observed clinically daily for 2 weeks. The vaccine is considered satisfactory if at least 80% of the chickens survive.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of vaccine by the recommended route. At 10 to 21 days post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged by inoculating ILT virus into the trachea or orbital sinus and observed for a minimum 10 days. At least 90% of the vaccinates must remain free from severe signs of ILT and 80% of controls should die or show signs.

5. VIRUS CONTENT

The vaccine should have a virus titre of not less than $10^{2.5}$ EID_{50} per dose when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR MAREK'S DISEASE VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as *Appendix 1*.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples.

2. PURITY TEST

Seedlot or bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods which appear as *Appendix 3*.

3. SAFETY TEST

Master seed should be tested as follows:

At least 20 susceptible, 1 day-old-chickens, are each inoculated with 10 doses of vaccine by the recommended route. At either 50 days or 120 days post-inoculation, all chickens are killed and examined histopathologically or grossly respectively for lesions of Marek's disease. No lesions of Marek's disease must be detected in any of the chickens.

4. VIRUS CONTENT

The vaccine should have a virus titre of not less than 1000 PFU per dose when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR MYCOPLASMA GALLISEPTICUM BACTERIN

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master and working seed bacteria should be *Mycoplasma gallisepticum* produced in a seed lot system. The seed bacteria must satisfy purity tests before they are used for bacterin production. The seed bacteria are lyophilized in small ampoules and stored at -18^0C or lower.

2. PRODUCTION SUBSTRATE

The medium should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of extraneous bacteria and fungi by the methods that appear as *Appendix 2*.

2. PURITY TEST

Bulk production samples should be tested as follows:

Live cultures are examined for morphological characteristics using Diene's stain. Only *M. gallisepticum* should be present.

3. INACTIVATION TEST

The bulk or final product is tested for inactivation in a medium known to support the growth of *M. gallisepticum* eg. Yeast infusion broth supplemented with protease peptone, yeast autolysate or fresh yeast extract and horse serum, with or without nicotinamide adenine dinucleotide and L-cysteine hydrochloride; PPLO (Hayflick) broth supplemented with horse serum and yeast extract.

Incubation is carried out at 33°C – 37°C for a minimum of 14 days. No *M. gallisepticum* or any other bacteria should be detected.

4. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with at least 1 dose of bacterin by the recommended route and observed for a minimum of 21 days. No abnormal local or systemic reaction attributable to the bacteria should occur in any of the chickens.

5. POTENCY TEST

Bulk or final container samples should be tested. At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each vaccinated as recommended. A minimum of 10 chickens are kept as unvaccinated controls. At least 2 weeks post-vaccination, the chickens are tested by one or more of the following:

a) The chickens are each serologically tested by the haemagglutination inhibition test. All controls should be negative and the vaccinates should have an average titre of at least 1/99.

b) The chickens are each challenged with virulent *M. gallisepticum* and observed for 14 days. At least 80% of the controls should show obvious clinical signs of *M. gallisepticum* infection and at least 80% of the vaccinates should remain free of clinical signs.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR NEWCASTLE DISEASE (LENTOGENIC STRAIN) VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

The pathotype of the seed virus should be identified by ICPI values or any other validated tests.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as *Appendix 1.*

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples.

2. PURITY TEST

Seedlot or bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods that appear as *Appendix 3*.

3. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 10 doses of the vaccine by the recommended routes. The chickens are observed clinically for 3 weeks. The

vaccine is considered satisfactory if all of the chickens do not show any adverse clinical signs of the disease.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows:

At least 20 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of the vaccine by the recommended routes. At least 2 weeks post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged with 10^4 LD_{50} or equivalent of a virulent Newcastle Disease Virus by the intramuscular route and observed for a minimum of 2 weeks post-challenge. At least 90% of the vaccinated chickens should remain healthy and show no clinical signs of the disease and at least 90% of the controls should die of Newcastle Disease.

5. VIRUS CONTENT

The vaccine should have a virus titre of at least $10^{5.5}$ EID_{50} per dose or 100 Minimum Protective Dose when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR NEWCASTLE DISEASE (MESOGENIC STRAIN) VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

The pathotype of the seed virus should be identified by ICPI values or any other validated tests.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as *Appendix 1*.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples.

2. PURITY TEST

Seedlot or bulk production should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods that appear as *Appendix 3*.

3. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 10 doses of the vaccine by the

recommended routes. The chickens are observed clinically for 3 weeks. The vaccine is considered satisfactory if 90% of the chickens do not show any adverse clinical signs of the disease.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows:

At least 20 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of the vaccine by the recommended routes. At least 2 weeks post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged with 10^4 LD_{50} or equivalent of a virulent Newcastle Disease Virus by the intramuscular route and observed for a minimum of 2 weeks post-challenge. At least 90% of the vaccinated chickens should remain healthy and show no clinical signs of the disease and at least 90% of the controls should die of Newcastle Disease.

5. VIRUS CONTENT

The vaccine should have a virus titre of at least $10^{4.0}$ EID_{50} per dose or 100 Minimum Protective Dose when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR NEWCASTLE DISEASE VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as *Appendix 1* or healthy flocks.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, fungi, Salmonella, and Mycoplasma by the methods that appear as Appendix 2**.** However, tests for Salmonella and Mycoplasma may be carried out on bulk samples. The test for Mycoplasma may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods which appear as Appendix 3. This test may be omitted if it can be demonstrated that the inactivating agent inactivates avian leucosis viruses.

3. INACTIVATION TEST

At least 10 embryonated chickens eggs susceptible to Newcastle disease (ND) virus are each inoculated with 0.2 ml of the inactivated product by the allantoic sac routes. The eggs are incubated for a minimum of 7 days. One subculture is carried out. There should be no evidence of ND virus.

4. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 2 doses of vaccine by the recommended route and observed for a minimum of 14 days. No abnormal local or systemic reaction attributable to the vaccine should occur in any of the chickens.

5. POTENCY TEST

Bulk or final container samples should be tested by one or more of the following methods:

a. At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of vaccine by the recommended route. At least 14 days post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged with $10^{5.0}$ EID_{50} of virulent ND virus and observed for 10 days. At least 80% of the vaccinates should survive and show no clinical signs of the disease and at least 80% of the controls should die.

b. Three groups of 20 susceptible chickens, aged 21-28 days, are inoculated intramuscularly with volumes of vaccine equivalent to 1/25, 1/50 and 1/100 of a dose. All the vaccinated chickens are challenged by intramuscular inoculation with $10^{6.0}LD_{50}$ of virulent ND virus together with 10 unvaccinated controls at 17-21 days post-vaccination. The chickens are observed for 21 days. All controls should die within 6 days. The vaccine should contain at least 50 PD_{50} per dose.

c. At least 25 SPF chicks of the minimum age for which the vaccine is intended, are each inoculated with 1/50 dose by the recommended route. The haemagglutination inhibition test may be carried out on the serum of bird. If mean HI titre of the vaccinated group is equal to or greater than 1:16 and the unvaccinated group is equal to or less than 1:4, then challenge shall be unnecessary. If this serological response is not achieved in all birds, challenged test should be performed like item (b).

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR RIEMERELLA ANATIPESTIFER BACTERIN

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master seed culture should be *Riemerella anatipestifer* produced in a seed lot system. The seed bacteria must satisfy purity tests before they are used for bacterin production. They must be dispensed into separate containers and stored at lower than –18^{O}C.

2. PRODUCTION SUBSTRATE

The medium used should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of extraneous bacteria and fungi by the methods that appear as *Appendix 2*.

2. PURITY TEST

Bulk production samples should be tested as follows:
Gram stained smears of live cultures are examined for morphological characteristics. Only *R. anatipestifer* should be present.

3. INACTIVATION TEST

The bulk or final product is tested for inactivation by culturing in a medium known to support growth of *R. anatipestifer*. No *R. anatipestifer* or any other bacteria should be detected.

4. SAFETY TEST

Final container samples should be tested as follows:
At least 10 susceptible ducklings are each inoculated with at least 2 doses of vaccine by the recommended route and observed for a minimum of 14 days. No abnormal local or systemic reaction attributable to the bacterin should occur in any of the ducklings.

5. POTENCY TEST

Bulk or final container samples should be tested as follows: Susceptible ducklings are each vaccinated with the recommended dose and route. At least 1 week after the final vaccination, the ducklings are divided into groups with at least 20 vaccinates and 10 unvaccinated controls per group for each serotype of *R. anatipestifer* contained in the bacterin. Each group is challenged with a virulent homologous serotype of *R. anatipestifer* (local strain) and observed for a minimum 10 days. At least 80% of the vaccinates should be protected, and at least 80% of the controls should succumb.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR VIRAL ARTHRITIS VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Embryonated eggs used throughout the production of the vaccine must be derived from SPF flocks complying with tests that appear as *Appendix 1*.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples.

2. PURITY TEST

Seedlot or bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and by the test for Avian Leucosis Virus subgroups A and B using the methods that appear as *Appendix 3*.

3. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 10 doses of the vaccine by the recommended routes. The chickens are observed clinically for 3 weeks. The

vaccine is considered satisfactory if all of the chickens do not show any adverse clinical signs of the disease.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows by one of the following methods:

a) At least 20 susceptible chickens of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of the vaccine by the recommended routes. At least 2 weeks post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged with virulent Viral Arthritis virus by inoculation into one foot pad and observed for a minimum of 2 weeks post-challenge. At least 90% of the vaccinated chickens should not show swelling and discolouration in the phalangeal joint area of the injected foot pad and at least 90% of the controls should show these lesions.

b) At least 20 susceptible of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of the vaccine by the recommended route. Ten unvaccinated chickens are used as control. Four weeks post-vaccination, the sera of vaccinated and control chickens are collected. Antibody titres are examined by Virus Neutralization Test (VN Test) with 100 $TCID_{50}$ of Viral Arthritis virus. The vaccine is considered satisfactory if Geometrical Mean Titer (GMT) of vaccinated chickens is at least 1:40 and GMT of control chickens should not be more than 1:4.

5. VIRUS CONTENT

The vaccine should have a virus titre of at least $10^{2.0}$ $TCID_{50}$ per dose when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR ACTINOBACILLUS PLEUROPNEUMONIAE BACTERIN

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master seed culture should be *Actinobacillus pleuropneumoniae* virulent for the intended species. They must be dispensed into separate containers and stored at lower than –18°C.

2. PRODUCTION SUBSTRATE

The medium used should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Mycoplasma and fungi by methods that appear as *Appendix 2*.

2. PURITY TEST

Bulk production samples should be tested as follows :

Gram stained smears of live cultures are examined for morphological characteristics. Only *A. pleuropneumoniae* should be present.

3. INACTIVATION TEST

The bulk product is tested for inactivation by culturing in a medium known to support the growth of *A. pleuropneumoniae* (e.g. blood agar cross-streaked with *Staphylococus aureus* or chocolate agar). No *A. pleuropneumoniae* or any other bacteria should be detected.

4. SAFETY TEST

Final container samples should be tested by one of the following methods:

a. At least 10 mice between 18-22 g are each inoculated with 0.5 ml of the bacterin by the intraperitoneal or subcutaneous route and observed for a minimum of 14 days. No unfavourable reaction should occur in the mice.

b. At least 2 guinea pigs are each inoculated with 2 ml of the bacterin by the subcutaneous or intramuscular route and observed for a minimum of 14 days. No unfavourable reaction attributable to the product should occur in the guinea pigs.

5. POTENCY TEST

Bulk or final container samples should be tested with one of the following methods :

a) Healthy mice are each inoculated with not more than 1/40 of the pig dose of the bacterin by the intraperitoneal route. At least 13 days later the mice may be re-inoculated as above. At least 10 days after the final inoculation the mice are divided into groups of 20 vaccinates and 20 unvaccinated controls per group for each serotype of *A.pleuropneumoniae* contained in the bacterin. Each group is challenged with a virulent homologous serotype of *A.pleuropneumoniae* and observed for a minimum of 10 days. At least 80% of the control should die and at least 75% of the vaccinates should survive. This test shall only be valid if a study had been carried out to establish the correlation between the mouse-dose and the pig-dose.

b) One mouse dose, equivalent to 1/6 of the pig dose of the test bacterin and a Standard Reference Bacterin (Standard) is prepared. At least 16 mice per group are each inoculated by the intraperitoneal route with a decreasing mouse dose of the bacterin and the Standard, respectively. At least 2 weeks later the mice are re-inoculated as above. At least 6 days after the final inoculation the vaccinates together with 10 unvaccinated controls are challenged with virulent *A.pleuropneumoniae* and observed for a minimum of 10 days. The relative potency of the test bacterin is compared with the Standard used. This test shall only be valid if a study had been carried out to correlate relative potency value to pig protective doses.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR AUJESZKY'S DISEASE VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The master and working seed viruses are produced in primary cells or cell lines in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Primary cells or cell lines used throughout production of the vaccine should be free from cytopathogenic and haemadsorbing agents. Porcine cells should be tested by the fluorescent antibody technique for absence of Swine Fever (SF) virus except where the vaccine is manufactured entirely in a country free from SF.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by methods that appear as *Appendix 2*. Test for Mycoplasma may be carried out in bulk samples.

2. PURITY TEST

Seedlot, bulk or final container samples should be tested for absence of extraneous viral agents by the Tissue culture inoculation test in Appendix 3.

3. SAFETY TEST

Final container samples should be tested by one or more of the following methods:

a) At least 5 mice are each inoculated with 0.5 ml of the vaccine by the intraperitoneal or subcutaneous route or 0.03 ml by the intracerebral route and observed for a minimum of 7 days. No unfavourable reaction attributable to the vaccine should occur in any of the mice.

b) At least 2 guinea pigs are each inoculated with 2 ml of the vaccine by the intraperitoneal, intramuscular or subcutaneous routes and observed for a minimum of 7 days. No unfavourable reaction attributable to the vaccine should occur in any of the guinea pigs.

c) At least 2 susceptible pigs are each inoculated with 10 doses of vaccine by the recommended route and observed for a minimum of 21 days. No clinical signs or lesions of any disease attributable to the vaccine should occur in any of the pigs.

4. POTENCY TEST

Seed/bulk/final container samples should be tested by one of the following methods:

a) At least 5 susceptible pigs are each vaccinated as recommended. At 14 to 28 days post-vaccination, the vaccinates together with 5 unvaccinated controls are each challenged with virulent Aujeszky 's disease (AD) virus and observed for 14 days. At least 80% of the controls should die or manifest clinical signs of AD and all vaccinates should survive and show no severe clinical signs.

b) Carry out a potency test for each of the routes of administration stated on the label. Use at least 5 susceptible pigs, weighing 15 to 35 kg, that are free from antibodies against AD virus or against a fraction of the virus. The body weight of the pigs should not differ from the average body weight of the group by more than 25%. Inject each pig by the route stated on the label with one dose of the vaccine. Use 5 similar pigs as control animals. Three weeks later, weigh each pig and challenge them by the intranasal route with a suitable quantity of a virulent strain of AD virus. Weigh each animal 7 days after challenge or at the time of death if this occurs earlier and calculate the average daily gain as a percentage. For each group (vaccinated and control animals) calculate the average of the average daily gains. The vaccinated pigs should survive and the difference between the average of the daily gains for the 2 groups should not be less than 1.6. The test is not valid unless all the control pigs display signs of AD and the average of their daily gains is less than –0.5.

5. VIRUS CONTENT

If potency test is not carried out on final container samples, the vaccine should be titrated. The vaccine should have a titre of not less than $10^{2.3}$ $TCID_{50}$ per dose when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR AUJESZKY'S DISEASE VACCINE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The master and working seed viruses are produced in primary cells or cell lines in a seedlot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Primary cells or cell lines used throughout production of the vaccine should be free from cytopathogenic and haemadsorbing agents. Porcine cells should be tested by the fluorescent antibody technique for absence of Swine Fever (SF) virus except where the vaccine is manufactured entirely in a country free from SF.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Mycoplasma and fungi by methods that appear as *Appendix 2*. However, tests for Mycoplasma may be carried out on bulk samples, but may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of cytopathogenic and haemadsorbing agents.

3. INACTIVATION TEST

The inactivated product should be tested by one or more of the following:

a) The inactivated product is passaged twice in cell cultures susceptible to Aujeszky's Disease (AD) virus. Incubation is for a minimum of 5 days per passage. There should be no evidence of AD virus.

b) At least 10 mice are each inoculated with 0.5 ml of the inactivated product by the subcutaneous or intraperitoneal route or 0.03 ml by the intracerebral route and observed for a minimum of 14 days. There should be no deaths or clinical signs of AD in any of the mice.

c) At least 3 rabbits are each inoculated with 2 ml of the inactivated product by subcutaneous or intramuscular route and observed for a minimum of 14 days. There should be no deaths or clinical signs of AD in any of the rabbits.

4. SAFETY TEST

Final container samples should be tested by one or more of the following:

a) At least 10 mice are each inoculated with 0.5 ml of the vaccine by the intraperitoneal or subcutaneous route and observed for a minimum of 7 days. No unfavourable reaction attributable to the vaccine should occur in any of the mice.

b) At least 2 guinea pigs are each inoculated with 2 ml of the vaccine by the intramuscular or subcutaneous route and observed for a minimum of 7 days. No unfavourable reaction attributable to the vaccine should occur in any of the guinea pigs.

c) At least 2 susceptible pigs are each inoculated with at least 1 dose of vaccine by the recommended route and observed for a minimum of 14 days. No clinical signs or lesions of any disease attributable to the vaccine should occur in any of the pigs.

5. POTENCY TEST

Bulk or **f**inal container samples should be tested by one or more of the following:

a) At least 5 susceptible pigs are each vaccinated as recommended. At least 14 days after the final vaccination, the vaccinates together with 5 unvaccinated controls are each serologically tested by the microneutralisation test. All controls should have titres less than 1: 2 and at least 80% of the vaccinates should have titres of at least 1: 8.

b) At least 5 susceptible pigs are each vaccinated as recommended. At least 14 days after the final vaccination the vaccinates together with 5 unvaccinated controls are each challenged with virulent AD virus and observed for 14 days. At least 80% of the controls should die or manifest clinical signs of AD and all vaccinates should survive and show no severe clinical signs.

c) For each route stated on the label, use at least 5 pigs, weighing 15 to 35 kg, that are free from antibodies against AD virus. All the pigs should be within 25% of the average body weight of the group. Inject each pig with 1 dose of the vaccine by the recommended route. Use 5 similar pigs as control animals. Three weeks later, weigh each pig and then challenge by the intranasal route with a suitable quantity of a virulent strain of AD virus. Weigh each animal 7 days after challenge or at the time of death if this occurs earlier and calculate the average daily gain as a percentage. For each group (vaccinated and control animals) calculate the average of the average daily gains. The vaccinated pigs should survive and the difference between the averages of the daily gains for the two groups should not be less than 1.6. The test is not valid unless all the control pigs display signs of AD and the average of their daily gains is less than –0.5.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR BORDETELLA BRONCHISEPTICA BACTERIN

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master seed culture should be *Bordetella bronchiseptica* for the intended species. The working seed bacteria shall satisfy purity and safety tests before they are used for vaccine production. They must be dispensed into separate containers and stored at lower than –18°C.

2. PRODUCTION SUBSTRATE

The medium used should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Mycoplasma and fungi by the methods that appear as *Appendix 2.*

2. PURITY TEST

Bulk production samples should be tested as follows:

Gram stained smears of live cultures are examined for morphological characteristics. Only *B. bronchiseptica* should be present.

3. INACTIVATION TEST

The bulk or final product is tested for inactivation by culturing in a medium known to support growth of *B. bronchiseptica.* No *B. bronchiseptica* or any other bacteria should be detected.

4. SAFETY TEST

Final container samples should be tested with one of the following methods:

a) At least 10 mice, 18-22g, are each inoculated with 0.5 ml of the bacterin by the intraperitoneal or subcutaneous route and

observed for a minimum of 14 days. The mice should not succumb.

b) At least 2 guinea pigs are each inoculated with 2 ml of the bacterin by the subcutaneous route and observed for a minimum of 14 days. No unfavourable reaction attributable to the product should occur in the guinea pigs.

5. POTENCY TEST

Bulk or final container samples should be tested by one of the following methods:

a) One mouse dose, equivalent to 1/6 pig dose, of the test bacterin and a Standard Reference Bacterin (Standard) is prepared. At least 16 mice per group are each inoculated by the intraperitoneal route with decreasing dilutions of the mouse dose of the bacterin and standard, respectively. At least 14 days later, the mice may be re-inoculated as above. At least 6 days after the final inoculation, the mice together with 10 unvaccinated controls are challenged with virulent *B. bronchiseptica* and observed for a minimum of 10 days. All controls should die.

The relative potency of the bacterin:

$$\frac{\text{Reciprocal of 50\% endpoint dilution of test bacterin}}{\text{Reciprocal of 50\% endpoint dilution of test Standard}}$$

should be at least 0.5.

b) Active Mouse Protection Test
A hundred mice are equally allocated into 2 groups. The first group is inoculated with 0.2 ml of the vaccine intraperitoneally. The second group serves as control. Three weeks post-vaccination, both groups are challenged with virulent *B. bronchiseptica*. The culture is diluted in ten-fold dilutions. A group of 5 mice are each inoculated with 0.1 ml of each culture dilution intraperitoneally. Seven days post-inoculation, LD_{50} of both groups are calculated. LD_{50} of the vaccination group should be higher than that of the control group by at least $10^{2.0}$.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR FOOT-AND-MOUTH DISEASE VACCINE FOR PIGS, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master seed virus should be selected, characterized and distributed by the official control laboratories in accordance with the epidemiological importance of each variant that has been adapted for growth in suspension or monolayer cells. The virus must satisfy sterility, purity and safety tests before they are used for vaccine production. Seed viruses must be tested by typing, subtyping and homology with the control virus. Seed viruses must be stored at –18°C if glycerinated or at lower temperature if not glycerinated.

2. PRODUCTION SUBSTRATE

Suspension or monolayer cells used throughout the production of the vaccine should be free from cytopathogenic and haemadsorbing agents.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by methods that appear as *Appendix 2.* Test for Mycoplasma may be carried out in bulk samples.

2. PURITY TEST

Bulk container samples should be tested for absence of cytopathogenic and haemadsorbing agents.

3. SAFETY TEST

Inoculate each of 2 healthy sero-negative pigs via:

a) the intramuscular route behind the ear with 2 doses of vaccine
b) the intracoronary route, into the left fore foot with a total of 1 dose of vaccine at 2 sites and
c) the prescribed route with 2 doses of vaccine.

Observe the inoculated pigs and 2 contact control pigs for 7 days. The animals should not present clinical signs and any foot-and-mouth disease (FMD) lesions in the foot, tongue or snout. A local reaction in the foot due to

the nature of the adjuvant is normal, but the animals should not present local reactions in the neck, nor general reactions.

4. POTENCY TEST

At least 5 healthy pigs that have not previously been vaccinated against FMD and that are free from antibodies neutralizing the different types of FMD virus, should be used. Vaccinate each animal with 1 dose by the route stated on the label. Three weeks after vaccination, challenge the vaccinated animals and 2 control animals with a suspension of swine virus that is fully virulent and of the same type or subtype as that used for the preparation of the vaccine, by inoculating 100 ID_{50} of virus, 0.5 ml each, into 2 sites of the coronary band of the external claw in 1 leg. Observe the animals for at least 4 days and then kill them. Animals that show lesions on un-inoculated legs are considered unprotected. The test is not valid unless control animals show generalizing lesions on legs not inoculated. The protected animals do not present any generalizing lesions in the 3 un-inoculated legs. The vaccine should protect at least 60% of the vaccinated pigs.

II. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR SWINE E. COLI BACTERIN

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master and working seed bacteria should be produced in a seed lot system. The seed bacteria must satisfy purity tests before they are used for bacterin production. They must be dispensed into separate containers and stored at lower than –18°C.

2. PRODUCTION SUBSTRATE

The medium should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Mycoplasma and fungi by methods that appear as *Appendix 2*.

2. PURITY TEST

Bulk Production samples should be tested as follows:
Gram stained smears of live cultures are examined for morphological characteristics. Only *E. coli* should be present.

3. INACTIVATION TEST

The bulk or final product is tested for inactivation by culturing in a medium known to support growth of *E. coli*. No *E. coli* or any other bacteria should be detected.

4. SAFETY TEST

Final container samples should be tested by one or more of the following methods :

a) At least 5 mice are each inoculated with 0.5 ml of the vaccine by the intraperitoneal or subcutaneous route and observed for a minimum of 7 days. No unfavourable reaction attributable to the vaccine should occur in any of the mice.

b) At least 2 guinea pigs are each inoculated with 2 ml of the vaccine by the intraperitoneal route and observed for minimum of 7 days. No intraperitoneal reaction attributable to the vaccine should occur in any of the guinea pigs.

c) At least 2 guinea pigs are each inoculated with 2 doses of the vaccine by the recommended route and observed for a minimum of 7 days. No abnormal local reaction or clinical signs of disease attributable to the vaccine should occur in any of the guinea pigs.

5. POTENCY TEST

Bulk or final container samples should be tested as follows:

At least 20 healthy, 6 to 7 weeks old mice, are each inoculated with not more than 1/10 dose of the vaccine by the recommended route. 3 to 4 weeks later, the mice may be re-inoculated as above. At least 10 days after the last inoculation the mice together with 10 unvaccinated controls are each serologically tested for antibodies against each antigenic component in the vaccine. The control should remain negative. There should be more than two-fold increase in the mean titre of mice inoculated once, while mice receiving two inoculations should have a mean titre greater than $10^{2.5}$. The test shall only be valid if a study has been carried out to establish the correlation of the test in mice and pigs.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR SWINE ERYSIPELAS BACTERIN

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

Master and working seed bacteria should be produced in suitable media in a seed lot system. The working seed bacteria shall satisfy purity and safety tests before they are used for bacterin production. They must be dispensed into separate containers and stored at lower than –18°C.

2. PRODUCTION SUBSTRATE

The medium should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Mycoplasma and fungi by the methods that appear as *Appendix 2.*

2. PURITY TEST

Bulk Production samples should be tested as follows:

Gram stained smears of live cultures are examined for morphological characteristics. Only *Erysipelothrix rhusiopathiae* should be present.

3. INACTIVATION TEST

The bulk or final product is tested for inactivation by culturing in a medium known to support growth of *E. rhusiopathiae*. No *E. rhusiopathiae* or any other bacteria should be detected

4. SAFETY TEST

Final container samples should be tested by one of the following methods:

a) Inject 2 healthy, susceptible pigs, 3 to 4 months old by the recommended route and with twice the recommended dose. Observe the animals for at least 10 days. No abnormal local or

systemic reaction attributable to the vaccine should occur in any of the pigs.

b) Inject 0.5 ml subcutaneously into healthy mice, each weighing 17-20 g. Observe the animals for at least 10 days. No abnormal local or systemic reaction attributable to the vaccine should occur in any of the mice.

5. POTENCY TEST

Bulk or final container samples should be tested by one or more of the following methods :

a) One mouse dose, equivalent to 1/10 pig dose, of the test bacterin and a Standard Reference Bacterin (Standard) are prepared. At least 20 mice per group are inoculated by the subcutaneous route with decreasing dilutions of the mouse dose of the bacterin and the Standard respectively. At least 14 days post-inoculation, all inoculated mice together with 10 uninoculated controls are challenged with virulent *E. rhusiopathiae* and observed for a minimum of 8 days. All controls should die.

The relative potency of the bacterin:

$$\frac{\text{Reciprocal of 50\% endpoint dilution of test bacterin}}{\text{Reciprocal of 50\% endpoint dilution of test Standard}}$$

should be at least 0.6.

b) Dilutions of the test bacterin and a Standard Reference calibrated in international units are prepared. At least 16 mice per group are each inoculated by the subcutaneous route with varying dilutions of the bacterin and the Standard respectively. At least 14 days post-inoculation, all inoculated mice together with 10 uninoculated controls are challenged with virulent *E. rhusiopathiae* and observed for a minimum of 8 days. All controls should die. The bacterin should, at confidence limit P=0.95, contain at least 50 IU per pig dose.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR SWINE FEVER VACCINE (CELL CULTURE ORIGIN), LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The master and working seed viruses are produced in primary cells or cell lines in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Primary cells or cell lines used throughout production of the vaccine should be free from cytopathogenic and haemadsorbing agents. Porcine cells should be tested by the fluorescent antibody technique for absence of Swine Fever (SF) virus except where the vaccine is manufactured in a country entirely free from SF.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. Test for Mycoplasma may be carried out in bulk samples.

2. PURITY TEST

Seedlot, bulk or final container samples should be tested for absence of extraneous viral agents by the Tissue culture inoculation test in Appendix 3.

3. SAFETY TEST

Final container samples should be tested by one or more of the following :

a) At least 5 mice are each inoculated with 0.5 ml of the vaccine by the intraperitoneal or subcutaneous route and observed for a minimum of 7 days. No unfavourable reaction attributable to the vaccine should occur in any of the mice.

b) At least 2 guinea pigs are each inoculated with 2 ml of the vaccine by the intraperitoneal route and observed for a

minimum of 7 days. No unfavourable reaction attributable to the vaccine should occur in any of the guinea pigs.

c) At least 2 susceptible pigs of the minimum age for which the vaccine is intended for are each inoculated with 10 doses of vaccine by the recommended route and observed for a minimum of 21 days. No clinical signs or lesions of any disease attributable to the vaccine should occur in any of the pigs.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows:

At least 4 susceptible pigs are each inoculated with 1/100 of a field dose of the product by the recommended route. After at least 2 weeks the pigs together with 2 unvaccinated controls are challenged with at least $10^{4.0}LD_{50}$ of virulent swine fever virus and observed for at least 14 days. The 2 controls must die or manifest serious signs of the disease. All vaccinates should survive and show no clinical signs of disease.

5. VIRUS CONTENT

If potency test is not carried out on final container samples, the vaccine should be titrated. The vaccine should have a titre of not less than $10^{2.3}$ $TCID_{50}$ per dose (or a virus titre equivalent to 100 pig protective doses) when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The virus strain should have been adapted, through serial passage in rabbits such that the virus has lost its virulence for swines of any age.

The master and working seed viruses are produced in healthy susceptible rabbits in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Healthy rabbits of any breed and having body weight of more than 2 kg shall be used for vaccine production.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of extraneous bacteria, fungi, Salmonella, and Mycoplasma by the methods that appear as Appendix 2. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples.

2. SAFETY TEST

Final container samples should be tested as follows:

At least 2 susceptible pigs of the minimum age for which the vaccine is intended for are each inoculated with 10 doses of vaccine by the recommended route and observed for a minimum of 21 days. No clinical signs or lesions of any disease attributable to the vaccine should occur in any of the pigs.

3. POTENCY TEST

Bulk or final container samples should be tested as follows:

At least 4 susceptible pigs are each inoculated with 1/100 of a field dose of the product by the recommended route. After at least 2 weeks the pigs together with 2 unvaccinated controls are challenged with at least $10^{4.0}LD_{50}$ of virulent swine fever virus and observed for at least 14 days. The 2

controls must die or manifest serious signs of the disease. All vaccinates should survive and show no clinical signs of disease.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as Appendix 4.

ASEAN STANDARD REQUIREMENTS FOR SWINE PASTEURELLA MULTOCIDA BACTERIN

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master seed culture should be *Pasteurella multocida* for the intended species. They must be dispensed into separate containers and stored at lower than –18°C.

2. PRODUCTION SUBSTRATE

The medium used should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Mycoplasma and fungi by methods that appear as Appendix 2.

2. PURITY TEST

Bulk production samples should be tested as follow:

Gram stained smears of live cultures are examined for morphological characteristics. Only *P. multocida* should be present.

3. INACTIVATION TEST

The bulk or final product is tested for inactivation by culturing in a medium known to support growth of *P. multocida*. No *P. multocida* or any other bacteria should be detected.

4. SAFETY TEST

Final container samples should be tested by one of the following methods:

a) At least 10 mice 18-22 g are each inoculated with 0.5 ml of the bacterin by the intraperitoneal or subcutaneous route and observed for a minimum of 14 days. None of the mice should show any adverse clinical signs.

b) At least 2 guinea pigs are each inoculated with 2 ml of the bacterin by the subcutaneous or intramuscular route and observed for a minimum of 14 days. No unfavourable reaction attributable to the product should occur in the guinea pigs.

5. POTENCY TEST

Bulk or final container samples should be tested by one of the following methods:

a) One mouse dose, equivalent to 1/20 pig dose, of the test bacterin and a Standard Reference Bacterin (Standard) is prepared. At least 10 mice per group are each inoculated by the mouse dose of the bacterin and Standard, respectively. At least 14 days later the mice may be re-inoculated as above. At least 10 days after the final inoculation, the mice are challenged with virulent *P. multocida* and observed for a minimum of 10 days. All controls should die.

The relative potency of the bacterin :

$$\frac{\text{Reciprocal of 50\% endpoint dilution of test bacterin}}{\text{Reciprocal of 50\% endpoint dilution of test Standard}}$$

Should be at least 0.5.

b) Active mouse protection test

A hundred mice are equally allocated into 2 groups. The first group is inoculated with 0.2 ml of the vaccine intraperitoneally. The second group serves as the control. Three weeks post-vaccination both groups are challenged with the vaccine strain of *P. multocida*. The challenged culture is diluted ten-fold. A group of 5 mice are each inoculated with 0.1 ml of each culture dilution intraperitoneally. Seven days post-inoculation, LD_{50} of both groups are calculated. LD_{50} of vaccination group must be higher than control group by at least $10^{2.0}$.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as Appendix 4.

ASEAN STANDARD REQUIREMENTS FOR ANTHRAX SPORE VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master seed culture should be Anthrax 34F2 Sterne strain obtained from WHO/FAO. They must be lyophilized or dispensed into separate containers and stored at -18°C or lower.

The vaccine should not be more than 3 passages from the master seed.

2. PRODUCTION SUBSTRATE

The medium used should contain artificial nutrients to allow optimal growth of the bacteria and promote sporulation of the organism.

II. QUALITY CONTROL REQUIREMENTS

1. PURITY TEST

Bulk production samples should be tested as follows:

Gram stained smears of live cultures are examined for morphological characteristics. Only *B. anthracis* should be present.

2. SAFETY TEST

Final container samples should be tested as follows:

Inject each of 2 sheep or goats subcutaneously with at least twice the dose stated on the label and observe the animals for at least 7 days. No abnormal systemic reactions or a progressive oedema should occur at the injection site.

3. VIABLE SPORE COUNT

Viable spore count shall be made by using Tryptose broth (TB) and Tryptose agar (TA). The culture shall be incubated at 37^{o}C for 20-24 hours. The recommended dose for cattle and horse is at least 2×10^{6} culturable spores.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR BRUCELLA ABORTUS VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

Master seed culture should be *Brucella abortus* strain 19 and are produced in suitable media in a seed lot system. The working seed bacteria shall satisfy sterility, purity and safety tests before they are used for vaccine production. The seed bacteria are ampouled or kept in small storage vials and stored at -18°C or lower.

2. PRODUCTION SUBSTRATE

The medium used should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of extraneous bacteria and fungi by the methods that appear as Appendix 2.

2. PURITY TEST

Bulk production samples should be tested as by one of the following:

a) Gram stained smears of culture are examined for morphological characteristics.
b) Inoculate on tryptose agar slopes, incubate at 37°C for 4 days and observe the characteristic appearance.
c) Inoculate a tube of dextrose Andrade's broth containing inverted ampoules. Incubate at 37°C and examined for fermentation characteristics at 2, 4 and 7 days.
d) Cells should be checked by agglutination test with antiserum to *Brucella* A antigen.

3. SAFETY TEST

Final container should be tested as follows:

At least 2 susceptible guinea pigs of weight 250 – 400 g, are each inoculated intramuscularly with 1/15 of the calf dose of vaccine in a volume of 1 ml and observed for a minimum of 10 days. No abnormal reactions should occur in the guinea pigs. The guinea pigs are killed on the 11^{th} day after inoculation, the blood is collected and the spleen is removed, weighed and cultures are prepared from it.

The spleen tissue of each animal must not contain more than 5×10^5 *Brucella* organisms/g, and the serum agglutinin titre with standard *B. abortus* antigen must not exceed 1,000 IU/ml.

4. POTENCY TEST

Final container samples should be tested as follows:

At least 12 guinea pigs or mice are each inoculated intramuscularly with 1/15 of the calf dose of vaccine in a volume of 1 ml. After the interval of not less than 8 weeks the vaccinated guinea pigs together with 6 unvaccinated control guinea pigs are challenged by the inoculation of 1 ml of a suspension containing 5×10^3 *B. abortus* organisms derived from virulent CO_2 dependent strain of known characteristics. After a further interval of not less than 6 weeks, the guinea pigs are killed and their spleen are weighed and emulsified separately, a separate suspension of each spleen being prepared in a suitable diluent, a volume of each suspension, equivalent to 0.05 g of the spleen, is inoculated on to a suitable medium and incubated at 37°C for 4 days.

B. abortus organisms of the challenge strain are not present in more than 25% of the suspension of the spleens of the vaccinated animals; the spleen of all unvaccinated control guinea pigs are infected and more than 80% of the unvaccinated control mice must die.

5. VIABLE COUNT

One dose of vaccine should contain between 40×10^9 and 120×10^9 viable organism.

6. DISSOCIATION TEST

Bulk production samples are tested for dissociation and should contain smooth colonies not less than 95%.

II. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR BLACKLEG BACTERIN

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

Master seed culture should be *Clostridium chauvoei* and are produced in suitable media in a seed lot system. The working seed bacteria shall satisfy sterility, purity and safety tests before they are used for vaccine production. The seed bacteria are ampouled or kept in small storage vials and stored at -18°C or lower.

2. PRODUCTION SUBSTRATE

The medium used should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of extraneous bacteria and fungi by the methods that appear as *Appendix 2.*

2. PURITY TEST

Bulk production samples should be tested as follow:

Gram stained smears of live cultures are examined for morphological characteristics. Only *C. chauvoei* should be present.

3. SAFETY TEST

Final container samples should be tested as follows:

a) At least 2 guinea pigs are each inoculated subcutaneously with 2 ml of the vaccine and observed for a minimum of 10 days. No unfavorable reaction attributable to the vaccine should occur in any of the guinea pigs.

b) Two healthy susceptible sheep are each inoculated subcutaneously with twice the dose stated on the label and

observed for a minimum of 7 days. No significant local or systemic reactions should occur.

4. POTENCY TEST

Bulk/final container samples should be tested by one of the following methods:

a) At least twenty 18-22 g mice are each inoculated 4 times intraperitoneally with 0.25ml of the vaccine at 2-day intervals. After a further interval of not less than 10 days, the vaccinated mice are challenged by the inoculation of a virulent culture of *C. chauvoei*. Not *less* than 60% of the vaccinated mice should survive for a minimum of 7 days after the challenge and more than 80% of the unvaccinated control mice must die.

b) At least 10 guinea pigs are injected subcutaneously with a quantity of the vaccine not greater than the minimum dose stated on the label as the primary dose. Twenty-eight days later vaccinate with a dose not greater than the minimum dose stated on the label as the secondary dose. After a further interval of 14 days, challenge each of the vaccinated guinea pigs, as well as each of no fewer than 5 control animals, by the intramuscular injection with 100 LD_{50} of a virulent culture or a spore suspension of *C. chauvoei* activated if necessary with a suitable activating agent such as calcium chloride. No unvaccinated guinea pigs should die from *C. chauvoei* infection within 5 days. All the control animals should die from *C. chauvoei* infection within 48 hours of challenge, or within 72 hours if a spore challenge is used. If one of the vaccinated animal dies, the test is repeated. None of the 2nd group of vaccinated guinea pigs should die from *C. chauvoei* infection within 5 days. All the 2nd group of control animals should die from *C. chauvoei* infection within 48 hours of challenge, or within 72 hours if a spore challenge is used.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR BOVINE VIRAL DIARRHOEA VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses should be shown to possess wide antigenicity diversity. The master and working seed viruses are produced in primary cells or cell lines in a seed lot system. The seed viruses must satisfy sterility, purity and safety tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Primary cells or cell lines used throughout production of the vaccine should be free from cytopathogenic and haemadsorbing agents.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Mycoplasma and fungi by methods that appear as Appendix 2. However, tests for Mycoplasma may be carried out on bulk samples, but may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of cytopathogenic and haemadsorbing agents.

3. INACTIVATION TEST

The inactivated product is passaged twice in cell cultures susceptible to BVD virus. Incubation is for a minimum of 5 days per passage. There should be no evidence of BVD virus.

4. SAFETY TEST

Final container samples should be tested as follows:

Vaccinates used in the potency test in this section shall be observed each day during the pre-challenge period. No abnormal local or systemic reactions attributable to the vaccine should occur in any calves.

5. POTENCY TEST

Bulk or final container samples should be tested as follows:

Eight healthy and susceptible calves (5 vaccinates and 3 controls) shall be used in this test. The 5 calves used as vaccinates shall be injected with 1 dose of vaccine as recommended. If 2 doses were recommended, the second dose shall be given according to the recommended interval. Fourteen days or more after the last vaccination, blood samples shall be collected and the individual serum samples are inactivated and tested for BVD antibody by a neutralizing antibody test or any other validated method. At least 4 of the 5 vaccinated calves should have a VN titre of 1: 8 or greater. If the results of a valid virus neutralization test or any other validated method are unsatisfactory, the vaccinates and controls should be challenged with virulent BVD virus. The animal shall be observed for 14 days post-challenge. At least 2 of the 3 control calves should show a temperature rise to 40°C and develop respiratory or clinical signs of BVD. No vaccinated animal or not more than 1 vaccinated animal should show a temperature rise to 40°C for 2 or more days and develop respiratory or clinical signs.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear *as Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR FOOT-AND-MOUTH VACCINE FOR CATTLE AND BUFFALOES, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master seed virus should be selected, characterized and distributed by the official control laboratories in accordance with the epidemiological importance of each variant that has been adapted for growth in suspension or monolayer cells. The virus must satisfy sterility, purity and safety tests before they are used for vaccine production. Seed viruses must be tested by typing, subtyping and homology with the control virus. Seed viruses must be stored at –18°C if glycerinated or at lower temperature if not glycerinated.

2. PRODUCTION SUBSTRATE

Suspension or monolayer cells used throughout the production of the vaccine should be free from cytopathogenic and haemadsorbing agents.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by methods that appear as Appendix 2. Test for Mycoplasma may be carried out in bulk samples.

2. PURITY TEST

Bulk container samples should be tested for absence of cytopathogenic and haemadsorbing agents.

3. SAFETY TEST

Final container samples should be tested as follows:

Use 3 non-vaccinated cattle, at least 6 months old, with serum free from Foot-and-Mouth disease (FMD) antibodies. Inoculate each animal intradermolingually at a minimum of 20 sites on the tongue using 0.1 ml of the vaccine at each site. Observe for not less than 4 days. No lesions of the disease should occur. At the end of the observation period, inject the animals by the same route with 3 times the dose stated on the label. Observe the

animals for 6 days after this inoculation. No lesions of the foot or tongue should occur and any reaction at the site of injection should remain small.

4. POTENCY TEST

Bulk or final container samples should be tested as follows:

Use unvaccinated cattle, not less than 6 months old, that are free from FMD neutralizing antibodies. Use an adjuvant-free buffer solution to prepare serial dilutions at 5-fold intervals. Vaccinate groups of at least 5 cattle by the recommended route, using one dilution per group. Three weeks after vaccination, challenge the vaccinated animals and 2 control animals with a suspension of virulent bovine FMD virus of the same type or subtype as that used for the preparation of the vaccine, by inoculating 10^4 ID_{50} intradermolingually into two sites on the upper surface of the tongue (0.1 ml per site). Observe the animals for 8 days and then kill them. Unprotected animals show lesions at sites other than the tongue. The test is not valid unless control animals show lesions on at least 3 feet. Protected animals may display lingual lesions. The vaccine should contain at least 3 PD_{50} per dose for cattle.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR HAEMORRHAGIC SEPTICAEMIA BACTERIN FOR CATTLE AND BUFFALOES

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master seed culture should be *Pasteurella multocida*, Carter type B, passaged in the natural ultimate host. They must be dispensed into separate containers and stored at –18°C or lower.

2. PRODUCTION SUBSTRATE

The medium used should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria and fungi by methods that appear as *Appendix 2.*

2. PURITY TEST

Bulk production samples should be tested as follow:

Gram stained smears of live cultures are examined for morphological characteristics. Only *P. multocida* should be present.

3. INACTIVATION TEST

The bulk or final product is tested for inactivation by culturing in a medium known to support growth of *P. multocida*. No *P. multocida* or any other bacteria should be detected.

4. SAFETY TEST

Final container samples should be tested by one or more of the following methods:

a) Ten mice between 18-22 g are each inoculated with 0.5 ml of vaccine subcutaneously, intraperitoneally or intramuscularly. The mice are observed for a minimum of 2 weeks. When there are no adverse reactions the batch is considered safe for use.

b) Two cattle are each inoculated with twice the recommended dose of vaccine by the recommended route. The cattle are observed for a minimum of 10 days. When there are no adverse reactions, the batch is considered safe for use.

c) Ten rabbits, 6-months old, are each inoculated with 0.5 ml of the vaccine, intramuscularly. The rabbits are observed for a minimum of 2 weeks. When there are no adverse reactions the batch is considered safe for use.

5. POTENCY TEST

Bulk or final container samples should be tested by one or more of the following methods :

a) Four of 5 cattle are each inoculated with 1 dose of vaccine by the recommended route, the other one is left as control. Four weeks post-vaccination, these cattle are challenged with 10^6 CFU of *P. multocida*, Carter type B, and observed for 7 days. The control cattle should die and all vaccinated cattle should survive and show no clinical symptoms.

b) Passive Mouse Protection Test (PMPT)
Five cattle are each inoculated with 1 dose of vaccine by the recommended route. Four weeks post-vaccination serum is collected. Thirty-five mice are divided into 5 groups. Five mice in each group are inoculated subcutaneously with 0.5 ml of serum collected from each cattle. The remaining mice represent serum controls. Another 5 mice represent challenge control. All mice are challenged 24 hours later with 100 LD_{50} of *P. multocida*, Carter type B, subcutaneously and observed for 7 days. All mice in the serum control group and challenge control group should die. The survival of 1/5 mice per group (20% protection) would be recorded as PMPT positive.

c) Three rabbits are each inoculated with 1 ml of the vaccine intramuscularly. Four weeks post-vaccination, sera are collected and pooled. 0.5 ml of the pooled serum is inoculated subcutaneously to each of 20 mice. Another 10 mice represent challenge control. All mice are challenged 24 hours later with 100 LD_{50} of *P. multocida*, Carter type B, subcutaneously and observed for 7 days. All controls should die and there should be at least 80% protection among the inoculated group for a batch to be taken as potent.

d) Active Mouse Protection Test

A hundred mice are equally allocated into 2 groups. Mice in the first group are each inoculated with 0.2 ml of the vaccine intraperitoneally. The second group acts as control. Three weeks post-vaccination, both groups are challenged with *P. multocida*, Carter type B. The culture is diluted in ten-fold dilutions. A group of 5 mice are each inoculated with 0.1 ml of each culture dilution intraperitoneally. Seven days post-inoculation, LD_{50} of both groups are calculated. The vaccine should give at least 10^4 units protection in the vaccinated mice.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR INFECTIOUS BOVINE RHINOTRACHEITIS VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The master and working seed viruses are produced in primary cells or cell lines in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Primary cells or cell lines used throughout production of the vaccine should be free from cytopathogenic and haemadsorbing agents.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. Test for Mycoplasma may be carried out in bulk samples.

2. PURITY TEST

Seedlot, bulk or final container samples should be tested for absence of extraneous viral agents by the Tissue culture inoculation test in *Appendix 3*.

3. SAFETY TEST

Final container samples should be tested as follows:

Inoculate each of 2 healthy susceptible calves, 2 to 6 months old, by the recommended route with 10 doses of the vaccine recommended on the label and observe for 21 days. No abnormal local and systemic reaction attributable to the product should occur in calves.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows:

Use 7 healthy susceptible calves, 2 to 3 months old and free from Infectious Bovine Rhinotracheitis (IBR) virus neutralizing antibodies.

Administer up to 5 calves by the recommended route on the label with a volume of the vaccine containing a quantity of virus equivalent to the minimum titre stated on the label. Keep the other 2 calves as control animals. After 21 days administer intranasally to the 7 calves a quantity of IBR virus sufficient to produce clinical signs of the disease. Observe the calves for 21 days. The vaccinated calves should show no more than mild signs and the control calves show typical signs of the disease. In at least 80% of the vaccinates, the maximum virus titre found in the nasal mucosa should be at least 100 times lower than the average of the maximum titres found in the control calves. The average number of days on which virus is excreted is at least 3 days less in the vaccinated calves than in the control calves.

5. VIRUS CONTENT

Determine the titre of vaccine in susceptible cell cultures at a temperature favourable to replication of the virus. Each dose of the vaccine should contain a quantity of virus that is equivalent or more than the minimum titre stated on the label and that confers protection in the conditions prescribed under the Potency Test.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR INFECTIOUS BOVINE RHINOTRACHEITIS VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed virus should be produced in suitable cell cultures in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Primary cells or cell lines used throughout production of the vaccine should be free from cytopathogenic and haemadsorbing agents.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Mycoplasma and fungi by methods that appear as Appendix 2. However, tests for Mycoplasma may be carried out on bulk samples, but may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of cytopathogenic and haemadsorbing agents.

3. INACTIVATION TEST

The inactivated product is passaged twice in cell cultures susceptible to Infectious Bovine Rhinotracheitis (IBR) virus. Incubation is for a minimum of 7 days per passage. There should be no evidence of IBR virus.

4. SAFETY TEST

Final container samples should be tested as follows:

Inoculate 2 healthy and susceptible calves, 3 to 6 month old, by the recommended route with twice the recommended dose and observe for 7 days. No abnormal local and systemic reactions attributable to the vaccine should occur in any of the calves.

5. POTENCY TEST

Bulk or final container samples should be tested as follows:

Vaccinate 5 healthy and susceptible calves free from IBR virus neutralizing antibodies by the recommended route and the recommended dose. Three weeks later, challenge all the calves together with 2 control animals intranasally with a virulent strain of IBR virus. Observe the animals for 14 days. No vaccinated animal should show more than a mild clinical reaction and all control animals should show typical signs of IBR infection.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR OVINE ECTHYMA (ORF) VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed viruses are produced in primary cells or cell lines in a seed lot system. The master and working seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at – 50°C or lower.

2. PRODUCTION SUBSTRATE

Primary cells or cell lines used throughout production of the vaccine should be free from cytopathogenic and haemadsorbing agents.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by methods that appear as *Appendix 2*. Test for Mycoplasma may be carried out in bulk samples.

2. PURITY TEST

Seedlot, bulk or final container samples should be tested for absence of extraneous viral agents by the Tissue culture inoculation test in *Appendix 3.*

3. SAFETY TEST

Final container samples should be tested as follows:

Each of 2 guinea pigs or 2 target ovine animals are inoculated either intramuscularly or subcutaneously with at least 2 ml doses of vaccine The guinea pigs are observed for 7 days. If ovine animals are used, the pre-challenge period of the potency test shall constitute a safety test. No abnormal local or systemic reactions attributable to the vaccine should occur in any of animals.

4. POTENCY TEST

Bulk or final container samples should be tested as follows:

Each of 2 susceptible lambs shall be vaccinated by application of the vaccine to a scarified area on the medial surface of the thigh and observed each day for 14 days. The immunity of the 2 vaccinates and 1 or more unvaccinated lambs (controls) shall be challenged in the same manner using the opposite thigh. Vaccinated animals should be protected against clinical manifestation of Ovine Ecthyma following challenge. An initial active reaction with hyperemia, which resolves progressively and disappears within 2 weeks, may be characterized as a typical immune reaction. If clinical signs of Ovine Ecthyma do not develop on the controls during the first 2 weeks following challenge and persist for approximately 30 days, the test is inconclusive and should be repeated.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR CANINE CONTAGIOUS HEPATITIS VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed virus should be produced in suitable cell cultures in a seed lot system. The seed viruses must satisfy sterility, purity and safety tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Primary cell culture or cell lines used throughout production of the vaccine should be free from cytopathogenic and haemadsorbing agents.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Mycoplasma and fungi by the methods that appear as Appendix 2. The test for Mycoplasma may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of cytopathogenic and haemadsorbing agents.

3. INACTIVATION TEST

Bulk or final product is tested by inoculating the inactivated product into cell culture. Several passages are carried out, during which no evidence of virus growth should be detected during an observation of 7 days.

4. SAFETY TEST

Final container samples should be tested as follows:

Inject subcutaneously 2 healthy and susceptible dogs, 8 to 14 weeks old, free from Canine Contagious Hepatitis (CCH) neutralizing antibodies, with

twice the dose recommended on the label. They shall be observed for 14 days and no unfavourable reaction attributable to the product should occur.

5. POTENCY TEST

Bulk or final container samples should be tested as follows:

Inject subcutaneously 2 healthy and susceptible dogs, 8 to 14 weeks old, free from CCH neutralizing antibodies, with the recommended dose. A second dose may be given 14 days later. Between 14 and 21 days after the last injection collect serum from each dog and examine each sample separately as follows: Heat the serum at 56°C for 30 minutes and prepare serial dilution in an incubation medium suitable for dog kidney cell cultures. Add to each dilution an equal volume of virus suspension containing an amount of virus such that when a volume of serum-virus mixture appropriate to the chosen assay system is subsequently inoculated into cell culture, each culture receives approximately 100 CID_{50}.

Incubate the mixture for 1 hour at 37°C and then inoculate 4 dog kidney cell cultures for 4 to 8 days at 37°C and examine for evidence of specific cytopathic effects. Calculate the 50% endpoint of the serum neutralization (SN) titre. Each dog should have a SN titre of at least 1: 80.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR FELINE PANLEUCOPENIA (FELINE INFECTIOUS ENTERITIS) VACCINE, LIVE

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The master and working seed viruses are produced in primary cells or cell lines in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Primary cells and cell lines used throughout the production of the vaccine should be free from cytopathogenic and harmadsorbing agents.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Salmonella, Mycoplasma and fungi by the methods that appear as Appendix 2. Test for Mycoplasma may be carried out in bulk samples.

2. PURITY TEST

Seedlot, bulk or final container samples should be tested for absence of extraneous viral agents by the Tissue culture inoculation test in *Appendix 3*.

3. SAFETY TEST

Final container samples should be tested as follows:

Inoculate 2 healthy, susceptible cats of the minimum age for which the vaccine is intended by the recommended route with 10 doses of vaccine. No abnormal local or systemic reaction should develop within 14 days of inoculation.

4. POTENCY TEST

Seed/bulk/final container samples should be tested as follows:

Inject 1 dose of the vaccine subcutaneously into 5 healthy susceptible cats, 2 to 4 months old. After 20 to 22 days, challenge the cats, together with 5 healthy susceptible controls, by the intraperitoneal inoculation of 0.5 ml of a suspension of pathogenic Feline Infectious Enteritis virus. Carry out leucocyte counts on the 4^{th} , 6^{th} , 8^{th} and 10^{th} days after the challenge. Each of the 5 control cats should have a mean leucocyte count that does not exceed 25% of its mean initial value established by 2 counts carried out respectively 8 days and 4 days before the inoculation of the viral suspension. These cats may die from leucopenia. When each of the 5 vaccinated cats shows no signs of leucopenia and has a mean leucocyte count that, on each of the four occasions, is not less than 50% of its mean initial value, these cats are considered to remain in excellent health.

5. VIRUS CONTENT

The virus content should not be less than 10^3 $CCID_{50}$ per dose when tested at any time before the expiry date.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear *as Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR FELINE PANLEUCOPENIA (FELINE INFECTIOUS ENTERITIS) VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master and working seed virus should be produced in suitable cell cultures in a seed lot system. The seed viruses must satisfy sterility, purity and safety tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Primary cell culture or cell lines used throughout production of the vaccine should be free from cytopathogenic and haemadsorbing agents.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria and fungi by the methods that appear in Appendix 2. The test for Mycoplasma may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of cytopathogenic and haemadsorbing agents.

3. INACTIVATION TEST

Bulk or final product is tested by inoculating the inactivated product into Feline cell culture. Several passages are carried out during which no evidence of virus growth should be detectable during an observation of 7 days.

4. SAFETY TEST

Final container samples should be tested as follows:

Inject subcutaneously 2 healthy and susceptible cats, by the recommended route with twice the recommended dose. Observe the cats for 14 days. Abnormal local and systemic reaction attributable to the significant local or systemic reaction should not occur.

5. POTENCY TEST

Bulk or final container samples should be tested using one of the following methods:

a) Use 4 healthy and susceptible cats, 8 – 12 weeks old, free from antibodies against feline enteritis virus. Inject by the recommended route with 1 dose of vaccine into 2 of the cats. After 21 days, serum is collected from all 4 cats. Inactivate each serum by heating at 56°C for 30 minutes. To one volume of each serum add 9 volumes of a 20% W/V suspension of light kaolin in phosphate buffered saline pH 7.4. Shake each mixture for 20 minutes. Centrifuge, collect the supernatant liquid and mix with 1 volume of concentrated suspension of pig erythrocytes. Allow to stand at 4°C for 60 minutes and centrifuge. The dilution of the serum obtained is 10-fold. Using each serum, prepare a series of 2-fold dilution. To 0.025 ml of each of the later dilutions, add 0.025 ml of a suspension of canine parvovirus antigen containing 4 haemagglutinating units. Allow to stand at 37°C for 30 minutes and add 0.05 ml of suspension of pig erythrocytes containing 30 X 10^6 cells per ml. Allow to stand at 4°C for 90 minutes and note the last dilution of serum that completely inhibits haemagglutination. The vaccine complies with the test if both vaccinated cats have developed titres of at least 1: 20. The test is not valid unless the control cats remain seronegative.

b) Use 2 healthy and susceptible cats, 8 – 12 weeks old, having neutralizing antibody titres less than 4 times of the neutralizing dose at 50% (ND_{50}) per 0.1 ml serum. Vaccinate by the schedule recommended on the label. Fourteen to 21 days after vaccination, examine the serum of each animal as follows: Heat the serum at 56°C for 30 minutes and prepare a serial dilution with an equal volume of virus suspension containing an amount of virus such that when the volume of serum–virus mixture appropriate for the assay system is inoculated into cell cultures, each culture receives approximately 10^4 $TCID_{50}$. Incubate the

mixtures of 37°C for 1 hour and inoculate 4 feline cells cultures with a suitable volume of each mixture. Incubate the cell cultures at 37°C for a 7-day passage and incubate for a further 7 days. Examine the cultures for evidence of specific cytopathic effect and calculate the antibody titre. The vaccine complies with the test if the mean titre is not less than 32 ND_{50} per 0.1 ml of serum. If 1 cat fails to respond, repeat the test using 2 more cats and calculate the result as the mean of the titre obtained from the 3 cats that have responded.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4*.

ASEAN STANDARD REQUIREMENTS FOR LEPTOSPIRA FOR DOGS, BACTERIN

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master seed culture should be *Leptospira* serotype(s) for the intended species. The working seed bacteria must satisfy purity and safety tests before they are used for vaccine production. They must be dispensed into separate containers and stored at lower than –18°C.

2. PRODUCTION SUBSTRATE

The medium used should contain artificial nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria and fungi by the methods that appear as *Appendix 2.*

2. PURITY TEST

Bulk production samples should be tested as follows:
Gram stained smears of live cultures are examined for morphological characteristics. Only *Leptospira spp.* should be present.

3. ABSENCE OF SERUM

lf serum has been used in the course of preparation of the vaccine, demonstrate its absence by carrying out a serum precipitation test.

4. INACTIVATION TEST

The bulk or final product is tested for inactivation by culturing in a medium known to support growth of *Leptospira spp.* No *Leptospira spp* or any other bacteria should be detected

5. SAFETY TEST

Final container samples should be tested as follows:

Use 2 healthy dogs susceptible to the serotype or serotypes with which the vaccine is prepared with twice the recommended dose by the recommended route. Observe the animals for 14 days. No significant local or systemic reactions should occur.

6. POTENCY TEST

Bulk or final container samples should be tested as follows:

Carry out a separate potency test for each serotype used to prepare the vaccine. Inject 5 healthy hamsters (not more than 3 months old and drawn from the same stock) subcutaneously with 1/10 of the dose of the vaccine recommended on the label for dogs. After 15 to 20 days, inject intraperitoneally into 5 vaccinated animals and into 5 controls an adequate dose of the virulent culture of the leptospirae of the serotype used to prepare the vaccine or a suspension of liver or kidney tissue obtained from animals infected with the serotype used to prepare the vaccine. At least 4 of the controls should die, showing typical leptospiral infection within 14 days of receiving the challenge suspension. At least 4 of the vaccinates should remain in good health for not less than 14 days after receiving the challenge suspension.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

ASEAN STANDARD REQUIREMENTS FOR RABIES VACCINE FOR DOGS AND CATS, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The seed should be any strain belonging to serotype 1 that has been proven to protect against field rabies viruses (currently found in the country where the vaccine is to be used). The working seed must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 10°C. Seed viruses in liquid form shall be stored at –50°C or lower.

2. PRODUCTION SUBSTRATE

Primary cell culture or cell lines used throughout production of the vaccine should be free from cytopathogenic and haemadsorbing agents.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Mycoplasma and fungi by the methods that appear as Appendix 2. The test for Mycoplasma may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of cytopathogenic and haemadsorbing agents.

3. INACTIVATION TEST

Final container samples should be tested by one of the following methods:

a) At least 10 suckling mice within 3 days of age are each inoculated intracerebrally with 0.01 ml of the vaccine. At least 10 other suckling mice are inoculated with the same volume of phosphate buffered saline intracerebrally. The animals are observed for at least 14 days. The vaccine shall be satisfactory if the experimental mice are free from symptoms caused by rabies

virus. If more than two animals die within the first 48 hours, the test should be repeated.

b) Tissue culture flasks are seeded with cell culture used for propagation of the virus and grown to near confluency. The inactivated viral suspension (2 ml) is then inoculated to the monolayer, and incubated for 60 minutes at 37°C. The inoculum is removed and the monolayer is maintained in medium containing 0.3% BSA for 14 days. At the end of the inoculation period, the cells are examined for rabies specific fluorescence. The test should be negative for florescence while the control should be positive for fluorescence.

4. SAFETY TEST

Final container samples should be tested as follows:

At least 2 guinea pigs weighing 350-400 g and at least 5 mice weighing 18-20 g are each injected intraperitoneally with 2 ml and 0.5 ml undiluted vaccine respectively. The animals are observed for at least 14 days. No abnormal symptoms shall be noticed during the observation period.

5. POTENCY TEST

Bulk/final container samples should be tested by one of the following methods:

a) Habel test;
b) NIH test.

The vaccine is satisfactory if it conforms to the requirements of the above tests.

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*

APPENDICES

STANDARD REQUIREMENTS FOR SPECIFIC-PATHOGEN-FREE (SPF) FLOCKS

GUIDELINES FOR STERILITY TESTING OF VETERINARY VACCINES

GUIDELINES FOR TESTING FOR EXTRANEOUS VIRUSES IN VETERINARY VACCINES

GENERAL REQUIREMENTS FOR VETERINARY VACCINES

APPENDIX 1

STANDARD REQUIREMENTS FOR SPECIFIC-PATHOGEN-FREE (SPF) FLOCKS

Specific-Pathogen-Free (SPF) flocks used to produce SPF embryonated eggs should be tested as follows:

a) 5% of flocks at monthly intervals and found to be free from the following pathogens; and

b) 50% at 3-months interval.

PATHOGEN	TEST
MYCOPLASMA **G**ALLISEPTICUM	SA
MYCOPLASMA **S**YNOVIAE	SA
SALMONELLA **P**ULLORUM	SA
NEWCASTLE DISEASE VIRUS	HI
INFECTIOUS BRONCHITIS VIRUS	HI/ ELISA/ AGPT
INFECTIOUS LARYNGOTRACHEITIS VIRUS	SN/ ELISA/ AGPT
IBD/INFECTIOUS BURSAL DISEASE VIRUS	SN/ ELISA/ AGPT
AVIAN ADENOVIRUS GP I	AGP/ SN/ FA
AVIAN ADENOVIRUS GP II	AGP
EDS 76 VIRUS	HI
FOWL POX VIRUS	CLINICAL/ AGP
AVIAN REOVIRUS	SN/ AGP/ FA
AVIAN ENCEPHALOMYELITIS VIRUS	FES/ ELISA/ AGP/ SN
MAREK' S DISEASE VIRUS	AGP
AVIAN LEUCOSIS VIRUS	PM/ SN/ ELISA
RETICULOENDOTHELIOSIS VIRUS	SN/ FA/ ELISA/ AGP
AVIAN INFLUENZA AGENT	AGP
OTHER SALMONELLAS	BACTERIAL EXAMINATION
CHICKEN ANAEMIA **V**IRUS	SN/ IFA/ ELISA
AVIAN NEPHRITIS VIRUS	FA
TURKEY RHINOTRACHEITIS VIRUS	ELISA

SA = Slide Agglutination
FES = Flock Embryo Susceptibility
PM = Phenotype Mixing

APPENDIX 2

GUIDELINES FOR STERILITY TESTING OF VETERINARY VACCINES

I. SAMPLE SIZE

The sample should consist of 1% of the number of containers in a batch subject to a minimum of 3 and a maximum of 10 containers.

II. TEST FOR BACTERIA

1. CULTURE MEDIA

a) Thioglycollate Broth, and
b) Soybean Casein Digest Broth, or
c) Trypticase Soy broth (TSB)

2. CULTURE CONDITION

The sample is reconstituted as recommended on the label and a minimum of 1 ml inoculated into any of the broth culture media to constitute not more than 10% of its volume. Incubation is at 30-37^{o}C for at least 14 days. TSB should also be incubated under anaerobic conditions.

In the case of non-parenteral biologics the sample is also inoculated onto Heart Infusion Agar.

INTERPRETATION

No bacterial contaminants should be detected, or in the case of non-parenteral biologics, not more than one saprophytic bacterial colony per dose should be detected.

II. TEST FOR MYCOPLASMA

1. CULTURE MEDIA

a) Heart Infusion Broth and Heart Infusion Agar with the following additives: proteose peptone, yeast autolysate or fresh yeast

extract, nicotinamide adenine dinucleotide, L-Cysteine hydrochloride, horse serum and tetrazolium chloride or other suitable indicator.

b) PPLO Broth and PPLO Agar with the following additives: horse serum and yeast extract.

2. CULTURE CONDITION

The sample is reconstituted as recommended and inoculated simultaneous into PPLO broth (1 ml in 100 ml) and PPLO agar (0.1 ml). The inoculated broth is incubated at 33-37^{o}C for 14 days, during which 0.1 ml is subcultured into a PPLO agar plate on the 3rd day, 7th day, 10th day and 14th day. All plates shall be incubated in a high humidity, 4-6% CO_2 atmosphere at 33-37^{o}C for 10-14 days.

INTERPRETATION

No mycoplasma contaminants should be detected in the agar plates by microscopic examination.

IV. TEST FOR SALMONELLA

1. CULTURE MEDIA

LIQUID MEDIA

a) Selenite Broth, or
b) Tetrathionate Broth, or
c) Rappaport Vassiliadis Broth (RV broth)

SOLID MEDIA

a) MacConkey Agar, or
b) Salmonella-Shigella Agar, or
c) Brilliant Green Agar, or
d) Desoxycholate Citrate Agar, or
e) XLD Agar, or
f) Gassner Agar, or
g) Rambach Agar, or
h) Bismuth Sulfite Agar

2. CULTURE CONDITION

The sample is reconstituted as recommended on label and minimum of 1 ml is inoculated into any of the liquid media to constitute not more than

10% of its volume. Incubation is at 35-37^{o}C for 18-24 hours and of which a loopful of the broth is inoculated onto any of the solid media. The solid medium is then incubated at 35-37^{o}C for at least 48 hours.

INTERPRETATION

No Salmonella contaminants should be detected.

V. TEST FOR FUNGI

1. CULTURE MEDIA

a) Sabouraud's Broth and Sabouraud's Agar, or
b) Soy bean Casein Digest Broth and Soybean Casein Digest Agar.

2. CULTURE CONDITION

The sample is reconstituted as recommended on the label and a minimum of 1 ml is inoculated into any of the broths to constitute not more than 10% of its volume. Incubation is at 20-25°C for 14 days. If necessary, the broth should be subcultured onto the corresponding agar and incubated for 14 days.

INTERPRETATION

No fungal contaminants should be detected.

APPENDIX 3

GUIDELINES FOR TESTING FOR EXTRANEOUS VIRUSES IN VETERINARY VACCINES

I. EGG INOCULATION TEST

The egg inoculation test should be carried out using the chorio-allantoic membrane (CAM) and allantoic sac (AS) routes of inoculation. The eggs must be SPF or known to be serologically negative to Newscastle Disease, CELO, Infectious Bronchitis, Marek's Disease, Egg Drop Syndrome 76, ***Mycoplasma gallisepticum***, ***Mycoplasma synoviae***, and ***Salmonella pullorum***.

1.1 CAM ROUTE

Ten field doses of neutralized vaccine are inoculated into 10 embryonated chicken eggs of age 9 to 12 days old by the CAM route. Dying embryos during the first 24 hours are discarded. All eggs that die after 24 hours and those that survive are examined for lesions in the CAMs and embryos. After 7 days, pooled CAMs are used to make further passage and the above procedure is repeated.

1.2 AS ROUTE

Ten field doses of neutralized vaccine are inoculated into 10 embryonated chicken eggs of age 9 to 12 days old by AS route. Dying embryos during the first 24 hours are discarded. All eggs that die after 24 hours and those that survive are examined for haemagglutinating activity. After 5-7 days, pooled allantoic fluid is used to make further passage and the above procedure is repeated.

INTERPRETATION

No deaths or abnormalities attributable to the vaccine should occur, or any haemagglutinating activity detected.

II. CHICKEN INOCULATION TEST

The chicken inoculation test should be carried out by one of the following methods :

2.1 At least 25 healthy chickens of the minimum age for which the vaccine is intended must be used. The chicken must be SPF or known to be serologically negative to Newcastle Disease, CELO, Infectious

Bronchitis, Marek's Disease, Egg Drop Syndrome 76, ***Mycoplasma gallisepticum***, ***Mycoplasma synoviae***, and ***Salmonella pullorum***. 20 chickens are inoculated with 10 doses of vaccine by each of the following routes: subcutaneous or intramuscular, comb scarification and eyedrop. 5 chickens are maintained as negative controls. The chickens are observed for 21 days for systemic and local reaction. Dead birds are subjected to post-mortem examination.

INTERPRETATION

The chickens should remain free from clinical signs attributable to the vaccine.

2.2 The test is carried out as above using 10 chickens. At the end of the observation period the chickens are serologically examined for presence of antibodies against all the agents listed above.

INTERPRETATION

The chickens should remain clinically healthy and serologically negative to all agents except the vaccine virus.

III. TISSUE CULTURE INOCULATION TEST

A sample of vaccine representing at least 10 field doses neutralized with monospecific hyperimmune serum against the vaccine is under test. The neutralized vaccine is then inoculated to at least one type of monolayer primary tissue culture or established cell line derived from a healthy animals species for which the vaccine is intended. Allow absorption to take place at 37^{o}C for one hour. Fresh tissue culture medium is added before reincubation at 37^{o}C. The monolayer cell is then observed for one week for cytopathic agents. If no cytopathic agent is detected, a blend of tissue culture fluid and cells is passaged once in fresh monolayer cell. If no specific cytopathic effect is detected, a haemadsorption test, using RBC from either guinea pig or from the animal species for which the vaccine is intended, is performed on the monolayer cell to detect any haemadsorbing agent. The vaccine is considered free of extraneous agent if no haemadsorbing agent is detected.

IV. TEST FOR AVIAN LEUCOSIS VIRUS

The chicken embryo fibroblast (CEF) cell cultures used must be susceptible to subgroups A and B of avian leucosis viruses. At least 10 doses of the neutralized vaccine are inoculated onto CEF (C/O or C/E) cultures. The cultures are maintained for a minimum of 3 weeks during which time the

cells are subcultured every 3-4 days. Positive control cultures using subgroup A and B viruses, and negative controls are included. At the end of the incubation period, the complement fixation test or a validated viral antigen detection test is carried out to demonstrate the absence of avian leucosis viral antigens.

INTERPRETATION

No avian leucosis viral contaminants should be detected.

APPENDIX 4

GENERAL REQUIREMENTS FOR VETERINARY VACCINES

I. VACUUM EXTENT TEST

Sample containers of freeze dried vaccines sealed under vacuum should be tested by using a high frequency vacuum tester. The vaccine is satisfactory if greenish blue fluorescence appears inside the container when the electrode is held close to it.

II. MOISTURE CONTENT

Final container sample of freeze dried vaccines should be tested for moisture content which must not exceed 4%.

III. HOMOGENICITY

3.1 Final containers of freeze dried vaccines must be a solid friable pellet and samples must be homogeneous after reconstitution with diluent.

3.2 Oil emulsion vaccines should be homogeneous after shaking.

IV. VACCINE STRAIN/ SEROTYPE

The strain incorporated in all vaccines should be stated, and if it is a new strain, the origin of the strain should be described and field trials carried out on experimental batches should be provided at registration.

V. LABELS AND INSTRUCTION LEAFLETS

Label or instruction leaflets should be included in the final containers. This should include the name of manufacturer, country of manufacture stating the lot or batch number, the strain(s) of vaccine, the intended animal species and their age, the number of doses, route of administration, the expiry date, the storage condition, precautions and other instructions on the proper handling and usage of the vaccine as well as vaccination schedules.

VI. SEED LOT SYSTEM

All vaccines should be produced from the vaccine seed which is not more than 5 passages from master seed.

VII. PRODUCTION ESTABLISHMENTS

Vaccines should be produced in establishments which are licensed by the veterinary authority of the country of manufacture for the production and report of the vaccine.

In the case of new establishments, detail information of the production and quality control facilities as well as personnel should be provided for registration of the establishment.

VIII. POTENCY TEST

Potency test should be carried out in target species, otherwise details of correlation test(s) should be carried out on the vaccine whenever a new master seed is used in the production of the vaccine.

IX. MINIMUM PROTECTIVE DOSE

Vaccines should indicate the minimum protective dose (MPD) and the acceptable MPD at date of opening of the vaccine

X. IDENTIFICATION TEST FOR MASTER SEED

The antigen used as vaccine should be identified as the specific organism using established validated identification methods.

XI. REVERSION TO VIRULENCE

For live attenuated virus vaccines, the seed should be tested by a minimum of 5 back passages in target animals for reversion to virulence, in animals for which the vaccine is intended.

XII. ADDITIONAL SAFETY TEST FOR INACTIVATED BACTERIAL AND VIRAL VACCINES FOR NON-AVIAN SPECIES

Bulk or final containers shall be tested for safety in young adult mice. If the product is inherently lethal for mice, the test shall be conducted in guinea pigs.

XIII. VALIDATION OF MASTER SEED IMMUNOGENICITY

The master seed should be validated every 3 years by demonstration of the minimum protective dose at the 5th passage level from the master seed.

XIV. CORRELATION OF VIRUS CONTENT WITH IN-VIVO IMMUNOGENICITY

Potency of virus content may substitute for the in-vivo potency test provided it is correlated to immunogenicity in the target species.

XV. POTENCY TEST BY SERO-CONVERSION

Potency tests based on in-vivo challenge in the target species may be substituted by sero-conversion provided that such serological values are correlated to immunogenicity in the target species.