Adopted at the 40th AMAF
11 October 2018
Ha Noi, Viet Nam

ASEAN Cooperation in Food, Agriculture and Forestry

ASEAN STANDARDS FOR ANIMAL VACCINES

Third Edition

Li v e s t o c k **P**ublication **S**eries **N**o.2A

2018

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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 master seed lot also complies with the test for extraneous agents in seed lot. In this test on the master seed lot, the organisms used are not more than 5 passages from master seed lot at the start of the test. 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

Seed lot 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 the chickens do not show notable clinical signs of disease or dies from causes attributable to the vaccine.

4. POTENCY TEST

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

a. Challenge Test
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.

b. Serum Neutralization Test
At least 10 SPF chickens of the minimum age for which the vaccine is intended, are each inoculated with 1 dose of vaccine by the recommended route. Ten unvaccinated SPF chickens, are kept as controls. Observe daily for 21 days. On the last day observation, collect the blood of both groups. Sera from vaccinated chickens are pooled, the sera from unvaccinated chickens are pooled, and then both pools of sera are inactivated in a water bath 56°C for 30 minutes. Serum neutralization test is performed using SPF egg, 9-10 days old. All eggs must be incubated in 37°C for 7 days. The neutralization index must not fewer than 2.0.

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.*

IV. REFERENCES

1. British Pharmacopoeia (Indonesia)

2. European Pharmacopoeia 8.0, 2013, Avian Infectious Bronchitis Vaccine (Live), 926-928.

3. Indonesian Vet Pharm., 2013 (full name? Indonesia)

4. OIE Terrestrial Manual 2013, Chapter 2.3.2 Avian Infectious Bronchitis.

ASEAN STANDARD REQUIREMENTS FOR INFECTIOUS BRONCHITIS VACCINE, INACTIVATED

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 master seed lot also complies with the test for extraneous agents in seed lot. In this tests on the master seed lot, the organisms used are not more that 5 passages from master seed lot at the start of the test. 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 by one or more of the following methods :

a. Haemagglutination Inhibition Test
   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 4 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 6 $\log_2$ and all controls should remain negative.

b. Serum Neutralization Test
   At least 10 SPF chickens the minimum age for which the vaccine is intended, are each inoculated with 1 dose of vaccine by the recommended route. Ten unvaccinated SPF chickens, are kept as controls. Two weeks after first vaccination, all vaccinates birds are boostered and observe for 2 weeks. On the last day of observation, collect the blood of both groups. Sera form vaccinated chickens are pooled, the serum from unvaccinated chickens are pooled, and then both pools of sera are inactivated in a water bath 56°C for 30 minutes. Serum neutralization test is performed using SPF egg, 9-10 days old. All eggs must be incubated at 37°C for 7 days. The neutralization index must not less than 2,0 (Indonesian Vet Pharm., 2013).

III. OTHER REQUIREMENTS

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

IV. REFERENCES

1. British Pharmacopoeia (Indonesia)

2. European Pharmacopoeia 8.0, 2013, Avian Infectious Bronchitis Vaccine (Live), 925-926.

3. Indonesian Vet Pharm., 2013 (full name? Indonesia)

4. OIE Terrestrial Manual 2013, Chapter 2.3.2 Avian Infectious Bronchitis.

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 master seed lot also complies with the test for extraneous agents in seed lot. In this tests on the master seed lot, the organisms used are not more that 5 passages from master seed lot at the start of the test. 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

Seed lot 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 the vaccine by the recommended routes. At least 2 weeks post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged with at least 10^4 EID (50% egg infectious dose) or 10^5 LD_{50} (50% lethal dose) 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*.

IV. REFERENCES

British Pharmacopoeia (Indonesia)

European Pharmacopoeia 8.0, 2013, Newcastle Disease Vaccine (Live), 997-999.

Indonesian Vet Pharm., 2013 (full name? Indonesia)

OIE Terrestrial Manual 2012, Chapter 2.3.14 Newcastle disease (Infection with Newcastle disease virus.

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 a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The master seed lot also complies with the test for extraneous agents in seed lot. In this tests on the master seed lot, the organisms used are not more that 5 passages from master seed lot at the start of the test. 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 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 2 weeks post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged with at least 10^4 EID (50% egg infectious dose) or 10^5 LD_{50} (50% lethal dose) 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.*

IV. REFERENCES

British Pharmacopoeia (Indonesia)

European Pharmacopoeia 8.0, 2013, Newcastle Disease Vaccine (Live), 997-999.

Indonesian Vet Pharm., 2013 (full name? Indonesia)

OIE Terrestrial Manual 2012, Chapter 2.3.14 Newcastle disease (Infection with Newcastle disease virus.

ASEAN STANDARD REQUIREMENTS FOR NEWCASTLE DISEASE VACCINE, INACTIVATED

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 master seed lot also complies with the test for extraneous agents in seed lot. In this tests on the master seed lot, the organisms used are not more that 5 passages from master seed lot at the start of the test. 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 14 – 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 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).

b. At least 10 susceptible chickens age 2-6 weeks, are each inoculated with ~~1 dose~~ minimum recommended dose of vaccine by the recommended route. At least 14 days post-vaccination, the vaccinates together with 10 unvaccinated controls are each challenged with $10^{4.0}$ CLD_{50} of virulent ND virus and observed for 14 days. At least 90% of the vaccinates should survive and show no clinical signs of the disease and 90% of the controls should die.

III. OTHER REQUIREMENTS

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

IV. REFERENCES

British Pharmacopoeia (Indonesia)

European Pharmacopoeia 8.0, 2013, Newcastle Disease Vaccine (Inactivated), 995-997.

Indonesian Vet Pharm., 2013 (full name? Indonesia)

OIE Terrestrial Manual 2012, Chapter 2.3.14 Newcastle disease (Infection with Newcastle disease virus.