**Monograph of Drugs**

**Vaccines**

**Akabane Disease Vaccine, Live, Seed**

1 Definition

Akabane Disease Vaccine, Live, Seed is a freeze-dried vaccine of virus suspension obtained by propagating attenuated Akabane virus that meets the Seed Lot Specifications in cell lines that meet the said specifications.

2 Production methods

2.1 Virus strain used for production

2.1.1 Name

Attenuated Akabane virus TS-C2 strain or strain approved as equivalent thereof

2.1.2 Properties

Inoculation of this virus strain into pregnant cows causes neither abortion, stillbirth, or congenital defects in newborns.

2.1.3 Master seed virus

2.1.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in HmLu-1 cells or cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -70℃ or lower or stored freeze-dried at 5℃ or lower.

The master seed virus shall be tested as specified in 3.1.1.

The master seed virus shall not be passaged for purposes other than production of vaccines. The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.4 Working seed virus

2.1.4.1 Propagation, passage and storage

The working seed virus shall be propagated and passaged in HmLu-1 cells or cells approved as suitable.

The working seed virus shall be stored frozen at -70℃ or lower or be stored freeze-dried at 5℃ or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.5 Production seed virus

2.1.5.1 Propagation and storage

The production seed virus shall be propagated in HmLu-1 cells or cells approved as suitable.

The production seed virus shall be stored frozen at -70℃ or lower or stored freeze-dried at 5℃ or lower, when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.2 Materials for production

2.2.1 Cell line

HmLu-1 cells or cell lines approved as suitable for production shall be used.

2.2.2 Culture medium

The culture medium approved as suitable for production shall be used.

2.2.3 Master cell seeds

2.2.3.1 Preparing, storing, and maximum passage number allowed for obtaining production cell seeds

The master cell seeds shall be propagated in a culture medium in 2.2.2, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master cell seeds shall be given a specific manufacturing number or manufacturing code and stored frozen at -70℃ or lower.

The master cell seeds shall be tested as specified in 3.2.1.

The master cell seeds shall not be passaged for purposes other than production or testing of vaccines. The master cell seeds shall not be passaged more than 20 times to obtain the production cell seeds.

2.2.4 Working cell seeds

2.2.4.1 Propagation, passage and storage

The working cell seeds shall be propagated and passaged using culture medium in 2.2.2.

The working cell seeds shall be stored frozen at -70℃ or lower. The working cell seeds shall be tested as specified in 3.2.2.

2.2.5 Production cell seeds

2.2.5.1 Propagation and storage

The production cell seeds shall be propagated using culture medium in 2.2.2.

The production cell seeds shall be stored frozen at -70°C or lower when it needs to be stored.

The production cell seeds shall be tested as specified in 3.2.3 if it needs to be stored.

2.3 Bulk material

2.3.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as an individual cultured cells. No abnormalities shall be detected prior to the inoculation of virus.

2.3.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.1. The filtrate or supernatant after centrifugation of culture medium collected from individual cultured cells at the peak of virus propagation shall be mixed, and the mixture shall serve as the bulk material.

The bulk material shall be tested as specified in 3.3.

2.4 Final bulk

A stabilizer approved as appropriate shall be added to the bulk material to serve as the final bulk.

2.5 Final product

The final bulk shall be dispensed into small containers and freeze-dried to serve as the final product.

The final product shall be tested as specified in 3.4.

3 Test methods

3.1 Tests on virus strain used for production

3.1.1 Tests on master seed virus

3.1.1.1 Identification test

The tests given in 1.4.2.1.1.1 in the Seed Lot Specifications shall apply.

3.1.1.2 Sterility test

The test given in the Sterility Test given of the General Tests shall apply.

3.1.1.3Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.1.4 Test for freedom from extraneous viruses

3.1.1.4.1 Common test for freedom from extraneous viruses

The tests given in 1.1, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.2 Test for freedom from specific viruses

3.1.1.4.2.1 General test for freedom from specific viruses

For Lymphocytic choriomeningitis virus, Bovine respiratory syncytial virus and Bluetongue virus, the tests given in 1.1 and 3.1.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.2.2 Individual test for freedom from specific viruses

For Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.1, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.5 Target animal immunogenicity test

The test given in the Target Animal Immunogenicity Test of the General Tests shall apply.

3.1.1.6 Target animal safety test

The test given in the Target Animal Safety Test of the General Tests shall apply.

3.1.1.7 Test for absence of reversion virulence

The test given in the Test for Absence of Reversion to Virulence of the General Tests shall apply.

3.1.2 Tests on working seed virus

3.1.2.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.2.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.3 Tests on production seed virus

3.1.3.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.3.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2 Tests on cell line

3.2.1 Tests on master cell seeds

3.2.1.1 Test for confirmation of cell properties

The test given in 2.1.4.2.1.1 in the Seed Lot Specifications shall apply.

3.2.1.2 Test for identification of the animal species of the cell

The test given in 2.1.4.2.1.2 in the Seed Lot Specifications shall apply.

3.2.1.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.1.4 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.1.5 Test for freedom from extraneous viruses

3.2.1.5.1 Common test for freedom from extraneous viruses

The tests given in 1.2, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.5.2 Test for freedom from specific viruses

3.2.1.5.2.1 General test for freedom from specific viruses

For Lymphocytic choriomeningitis virus, Bovine respiratory syncytial virus and Bluetongue virus, the tests given in 1.2 and 3.1.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.5.2.2 Individual test for freedom from specific viruses

For Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus and Rabies virus, the tests given in 1.2, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.6 Test for karyological (chromosomal) characterization

The test given in 2.1.4.2.1.6 in the Seed Lot Specifications shall apply.

3.2.1.7 Test for freedom from tumorigenicity

The test given in 2.1.4.2.1.7 in the Seed Lot Specifications shall apply.

3.2.2 Tests on working cell seed~~s~~

3.2.2.1 Test for confirmation of cell properties

The test given in 2.1.4.2.2.1 in the Seed Lot Specifications shall apply.

3.2.2.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.2.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.3 Tests on production cell seed~~s~~

3.2.3.1 Test for confirmation of cell properties

The test given in 2.1.4.2.3.1 in the Seed Lot Specifications shall apply.

3.2.3.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.3.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.3 Tests on bulk material

3.3.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.3.2 Test for virus content

3.3.2.1 Materials

3.3.2.1.1 Test materials

The test article shall be diluted ten-fold with virus growth medium (Note) and each level dilution shall serve as a test material.

3.3.2.1.2 Cultured cells

HmLu-1 cells shall be cultured in small test tubes for one to three days, and monolayer cultures shall be used.

3.3.2.2 Test procedures

A 0.1 mL portion of the test material shall be inoculated into at least four tubes of cultured cells and allowed to stand for adsorption at 37℃ for 60 minutes. A 0.5 mL portion of virus growth medium shall be added, roller-cultured at 34℃ to 36℃ for seven days and observed.

3.3.2.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 106.5TCID50 or higher.

3.4 Tests on final product

3.4.1 Properties test

When the test is performed as specified in the Properties Test of the General Tests, the final product shall be dried substances with a specific color. The dissolved product shall be a liquid with a specific color, and no foreign matter and foreign odor shall be observed. The property of product per small container shall be uniform.

3.4.2 Vacuum degree test

The test given in the Vacuum Degree Test of the General Tests shall apply.

3.4.3 Test for moisture content

The test given in the Test for Moisture Content of the General Tests shall apply.

3.4.4 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.4.5 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply. However, this test may be omitted if it is conducted during the intermediate process including the bulk material.

3.4.6 Test for virus content

When the test is performed as specified in 3.3.2, the virus content of the test sample shall be 105.0 TCID50 or higher per animal.

3.4.7 Test for freedom from abnormal toxicity

The test given in the Test for Freedom from Abnormal Toxicity of the General Tests shall apply.

3.4.8 Safety test

3.4.8.1 Materials

3.4.8.1.1 Injection materials

The test sample shall be used as an injection material.

3.4.8.1.2 Test animals

Cattle weighing 100–200 kg shall be used.

3.4.8.2 Test procedures

A dose of injection material for one animal shall be administered subcutaneously to one animal and observed for 14 days.

3.4.8.3 Judgment

During the observation period, a slight fever (40.5℃ or lower), if observed, shall not persist for more than three days, and no other abnormalities shall be identified.

3.4.9 Potency test

3.4.9.1Materials

3.4.9.1.1 Test animals

The animals used in the test specified in 3.4.8 shall be used.

3.4.9.1.2 Virus for neutralization test

Akabane virus JaGAr39 strain propagated in HmLu-1 cells shall be used.

3.4.9.1.3 Cultured cells

HmLu-1 cells shall be cultured in small test tubes for one to three days, and monolayer cultures shall be used.

3.4.9.2 Test procedures

The serum obtained 14 days after the completion of test specified in 3.4.8 shall be subject to the neutralization test.

The serum shall be heat-inactivated, and then shall be diluted two-fold with virus growth medium. With 0.5 mL of each diluted serum, 0.5 mL of the virus for the neutralization test with approximately 200 TCID50 per 0.1 mL shall be mixed and reacted at 37℃ for 60 minutes. The 0.1 mL of the mixtures of each shall be inoculated into four tubes of cultured cells and allowed for adsorption at 37℃ for 60 minutes. To each tube, 0.5 mL of virus growth medium shall be added and roller-cultured at 34℃ to 36℃ for seven days for observation.

3.4.9.3 Judgment

The maximum dilution factor of serum at which CPE is observed in at least two tubes of cultured cells shall be defined as the neutralizing antibody titer.

The neutralizing antibody titer in test animals shall be at least two-fold or higher.

4 Storage and expiry date

The expiry date shall be two years and three months after the manufacturing unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

Note Virus growth medium

1,000 mL consists of the following:

Tryptose phosphate broth 2.95 g

Glucose 1.0 g

Yeast extract 0.5 g

Bovine serum 5-20 mL

Eagle’s MEM residual quantity

pH shall be adjusted to 7.2-7.6 with sodium hydrogen carbonate.

Bovine serum to be used shall be negative for neutralizing antibodies against the Akabane virus.

Minimal amounts of antibiotics may be added.

**Bovine Respiratory Syncytial Virus Infection Vaccine, Live, Seed**

1 Definition

Bovine respiratory syncytial virus infection vaccine, live, seed is a freeze-dried vaccine of the virus suspension obtained by propagating attenuated Bovine respiratory syncytial virus that meets the Seed Lot Specifications in cell lines that meet the said specifications.

2 Production methods

2.1 Virus strain used for production

2.1.1 Name

Attenuated Bovine respiratory syncytial virus rs-52 strain or strain approved as equivalent thereof

2.1.2 Properties

This virus strain shows no pathogenic effects when inoculated in cows. The propagative ability of the strain at 30ºC in HAL cells established from cells derived from hamster lung is 100-fold higher than that of the virulent strain.

2.1.3 Master seed virus

2.1.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in HAL cells or cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.

The master seed virus shall not be passaged for purposes other than the production of vaccines.

The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.4 Working seed virus

2.1.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in HAL cells or cells approved as suitable. The working seed virus shall be stored frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.5 Production seed virus

2.1.5.1 Propagation and storage

The production seed virus shall be propagated in HAL cells or cells approved as suitable.

The production seed virus shall be stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.2 Materials for production

2.2.1 Cell line

HAL cells or cell lines approved as suitable for production shall be used.

2.2.2 Culture medium

The culture medium approved as suitable for production shall be used.

2.2.3 Master cell seeds

2.2.3.1 Preparing, storing, and maximum passage number allowed for obtaining production cell

seeds

The master cell seeds shall be propagated in a culture medium in 2.2.2, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master cell seeds shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower.

The master cell seeds shall be tested as specified in 3.2.1.

The master cell seeds shall not be passaged for purposes other than production or testing of vaccines. The master cell seeds shall not be passaged more than 20 times to obtain the production cell seeds.

2.2.4 Working cell seeds

2.2.4.1 Propagation, passage, and storage

The working cell seeds shall be propagated and passaged using culture medium in 2.2.2.

The working cell seeds shall be stored frozen at -70ºC or lower. The working cell seeds shall be tested as specified in 3.2.2.

2.2.5 Production cell seeds

2.2.5.1 Propagation and storage

The production cell seeds shall be propagated using culture medium in 2.2.2.

The production cell seeds shall be stored frozen at -70°C or lower when it needs to be stored.

The production cell seeds shall be tested as specified in 3.2.3 if they need to be stored.

2.3 Bulk material

2.3.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as individual cultured cells. No abnormalities shall be detected in production cell seeds prior to the inoculation of the virus.

2.3.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.1. The filtrate or supernatant after centrifugation of culture medium collected from individual cultured cells at the peak of the virus propagation shall be mixed, and the mixture shall serve as the bulk material.

The bulk material shall be tested as specified in 3.3.

2.4 Final bulk

A stabilizer approved as appropriate shall be added to the bulk material to serve as the final bulk.

2.5 Final product

The final bulk shall be dispensed into small containers and freeze-dried to serve as the final product.

The final product shall be tested as specified in 3.4.

3 Test methods

3.1 Tests on virus strain used for production

3.1.1 Tests on master seed virus

3.1.1.1 Identification test

The tests given in 1.4.2.1.1.1.2 in the Seed Lot Specifications shall apply.

3.1.1.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.1.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.1.4 Test for freedom from extraneous viruses

3.1.1.4.1 Common test for freedom from extraneous viruses

The tests given in 1.1, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.2 Test for freedom from specific viruses

3.1.1.4.2.1 General test for freedom from specific viruses

For the lymphocytic choriomeningitis virus and Bluetongue virus, the tests given in 1.1 and 3.1.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.1, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.5 Target animal immunogenicity test

The test given in the Target Animal Immunogenicity Test of the General Tests shall apply.

3.1.1.6 Target animal safety test

The test given in the Target Animal Safety Test of the General Tests shall apply.

3.1.1.7 Test for absence of reversion virulence

The test given in the Test for Absence of Reversion to Virulence of the General Tests shall apply.

3.1.2 Tests on working seed virus

3.1.2.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.2.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.3 Tests on production seed virus

3.1.3.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.3.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2 Tests on cell line

3.2.1 Tests on master cell seeds

3.2.1.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.1.1 in the Seed Lot Specifications shall apply.

3.2.1.2 Test for identification of the animal species of the cell

The tests given in 2.1.4.2.1.2 in the Seed Lot Specifications shall apply.

3.2.1.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.1.4 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.1.5 Test for freedom from extraneous viruses

3.2.1.5.1 Common test for freedom from extraneous viruses

The tests given in 1.2, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.5.2 Test for freedom from specific viruses

3.2.1.5.2.1 General test for freedom from specific viruses

For the Lymphocytic choriomeningitis virus, Bluetongue virus, and Bovine respiratory syncytial virus, the tests given in 1.2 and 3.1.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.5.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.2, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.6 Test for karyological (chromosomal) characterization

The tests given in 2.1.4.2.1.6 in the Seed Lot Specifications shall apply.

3.2.1.7 Test for freedom from tumorigenicity

The tests given in 2.1.4.2.1.7 in the Seed Lot Specifications shall apply.

3.2.2 Tests on working cell seeds

3.2.2.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.2.1 in the Seed Lot Specifications shall apply.

3.2.2.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.2.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.3 Tests on production cell seeds

3.2.3.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.3.1 in the Seed Lot Specifications shall apply.

3.2.3.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.3.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.3 Tests on bulk material

3.3.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.3.2 Test for virus content

3.3.2.1 Materials

3.3.2.1.1 Test materials

The test article shall be diluted ten-fold with the virus growth medium (Note 1) and each level dilution shall serve as the test material.

3.3.2.1.2 Cultured cells

Vero cells shall be cultured in small test tubes for three to four days, and monolayer cultures shall be used.

3.3.2.2 Test procedures

The 0.1 mL portions of the test material shall be inoculated into at least four portions of cultured cells and allowed to stand for adsorption at 37ºC for 60 minutes. The 0.5 mL portions of the virus growth medium shall be added, roller-cultured at 34ºC for 14 days, and observed.

3.3.2.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 106.3TCID50 or higher.

3.4 Tests on final product

3.4.1 Properties test

When the test is performed as specified in the Properties Test of the General Tests, the final product shall be dried substances with a specific color. The dissolved product shall be a liquid with a specific color, and no foreign matter and foreign odor shall be observed. The property of the product per small container shall be uniform.

3.4.2 Vacuum degree test

The test given in the Vacuum Degree Test of the General Tests shall apply.

3.4.3 Test for moisture content

The test given in the Test for Moisture Content of the General Tests shall apply.

3.4.4 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.4.5 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

However, this test may be omitted if it is conducted during the intermediate process, including the bulk materials.

3.4.6 Test for virus content

When the test is performed as specified in 3.3.2, the virus content of the test sample shall be 105.0 TCID50 or higher per animal.

3.4.7 Test for freedom from abnormal toxicity

The test given in the Test for Freedom from Abnormal Toxicity of the General Tests shall apply.

3.4.8 Safety test

3.4.8.1 Materials

3.4.8.1.1 Injection materials

The test samples shall be used as injection materials.

3.4.8.1.2 Test animals

Cattle weighing 100–200 kg shall be used.

3.4.8.2 Test procedures

A dose of injection material for one animal shall be administered intramuscularly to one animal and observed for 14 days.

3.4.8.3 Judgment

During the observation period, a slight fever (40.5ºC or lower), if observed, shall not persist for more than three days, and no other abnormalities shall be identified.

3.4.9 Potency test

3.4.9.1 Materials

3.4.9.1.1 Injection materials

The test samples shall be used as injection materials.

3.4.9.1.2 Test animals

Hamsters weighing approximately 100 g shall be used.

3.4.9.1.3 Virus for neutralization test

The Bovine respiratory syncytial virus NMK7 strain propagated in passage cells of bovine kidneys.

3.4.9.1.4 Cultured cells

Vero cells shall be cultured in small test tubes for three to four days, and monolayer cultures shall be used.

3.4.9.2 Test procedures

The 2 mL portions of the injection materials shall be injected intramuscularly twice at intervals of 14 days in five test animals, and serum shall be obtained from individual animals 14 days after the second injection to conduct a neutralization test.

The test serum shall be heat-inactivated and diluted with the dilution for neutralization test (Note 2) in two-fold serial dilutions. A 0.5 mL portion of diluted serum and a 0.5 mL portion of the virus suspension for neutralization test containing approximately 200TCID50 in 0.1 mL shall be mixed and reacted at 22ºC for 24 hours. The 0.1 mL portion of each mixture shall be inoculated into four tubes of cultured cells and allowed to stand for adsorption at 37ºC for 60 minutes. Then, the 0.5 mL portions of the virus growth medium shall be added, roller-cultured at 34ºC for 10 days, and observed.

3.4.9.3 Judgment

The maximum dilution factor of serum at which inhibition of CPE is observed in at least two tubes of cultured cells shall be defined as the neutralizing antibody titer. A two-fold or greater increase in the neutralizing antibody titer shall be defined as positive.

The neutralizing antibody positive rate in test animals shall be 80% or higher.

4 Storage and expiry date

The expiry date shall be one year and three months after the manufacturing unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

Note 1 Virus growth medium

1,000 mL consists of the following:

|  |  |
| --- | --- |
| Tryptose phosphate broth | 2.95 g |
| Monosodium glutamate | 5 g |
| Glucose | 1 g |
| Fetal bovine serum | 10–20 mL |
| Yeast extract | 0.5 g |
| Eagle’s MEM | Residual quantity |

The pH shall be adjusted to 7.2–7.6 with sodium hydrogen carbonate.

Fetal bovine serum to be used shall be negative for neutralizing antibodies against the bovine respiratory syncytial virus.

Minimal amounts of antibiotics may be added.

Note 2 Dilution for neutralization

1,000 mL consists of the following:

|  |  |
| --- | --- |
| Tryptose phosphate broth | 2.95 g |
| Fetal bovine serum | 100 mL |
| Kanamycin sulfate | 100 mg (potency) |
| Eagle’s MEM | Residual quantity |

The pH shall be adjusted to 7.2–7.4 with sodium hydrogen carbonate.

Fetal bovine serum to be used shall be negative for neutralizing antibodies against the bovine respiratory syncytial virus.

Minimal amounts of antibiotics may be added.

**Akabane Disease, Chuzan Disease, Aino Virus Infection, Peaton Virus Infection Vaccine (adjuvant), Inactivated, Seed**

1 Definition

This Vaccine, Inactivated, Seed is made by mixing the inactivated virus suspensions obtained by propagating the Akabane virus, Kasba virus, Aino virus and Peaton virus that meet the Seed Lot Specifications in cell lines that meet the said specifications, respectively, and by adding an adjuvant.

2 Production methods

2.1 Virus strain used for production

2.1.1 Akabane virus

2.1.1.1 Name

Akabane virus E-24 strain or strain approved as equivalent thereof

2.1.1.2 Properties

This virus strain shall be propagated in the following cells in which CPE is confirmed: primary cultured cells of bovine kidney, primary cultured cells of swine kidney, HmLu-1 cells, HmLu-SC cells, and Vero cells.

2.1.1.3 Master seed virus

2.1.1.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in HmLu cells or cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.

The master seed virus shall not be passaged for purposes other than the production of vaccines. The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.1.4 Working seed virus

2.1.1.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in HmLu cells or cells approved as suitable.

The working seed virus shall be stored frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.1.5 Production seed virus

2.1.1.5.1 Propagation and storage

The production seed virus shall be propagated in HmLu cells or cells approved as suitable.

The production seed virus shall be stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.1.2 Kasba virus

2.1.2.1 Name

Kasba virus K-47 strain or strain approved as equivalent thereof

2.1.2.2 Properties

Inoculation of this virus strain into the brain of calves causes fever, anorexia, and leukopenia, followed by neurological symptoms.

The strain shall be propagated in the following cells in which CPE is confirmed: BHK-21 cells, BHK-SC cells, HmLu-1 cells, HmLu-SC cells, and Vero-T cells.

2.1.2.3 Master seed virus

2.1.2.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in HmLu cells or cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.

The master seed virus shall not be passaged for purposes other than the production of vaccines. The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.2.4 Working seed virus

2.1.2.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in HmLu cells or cells approved as suitable.

The working seed virus shall be stored frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.2.5 Production seed virus

2.1.2.5.1 Propagation and storage

The production seed virus shall be propagated in HmLu cells or cells approved as suitable.

The production seed virus shall be stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.1.3 Aino virus

2.1.3.1 Name

Aino virus JaNAr28 strain or strain approved as equivalent thereof

2.1.3.2 Properties

When this virus strain is inoculated intravenously in cows, viremia is present, but no clinical symptoms such as fever are observed.

The strain shall be propagated in the following cells in which CPE is confirmed: BHK-21 cells, HmLu-1 cells, HmLu-SC cells, and Vero cells.

2.1.3.3 Master seed virus

2.1.3.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in HmLu cells or cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.

The master seed virus shall not be passaged for purposes other than the production of vaccines. The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.3.4 Working seed virus

2.1.3.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in HmLu cells or cells approved as suitable.

The working seed virus shall be stored frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.3.5 Production seed virus

2.1.3.5.1 Propagation and storage

The production seed virus shall be propagated in HmLu cells or cells approved as suitable.

The production seed virus shall be stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.1.4 Peaton virus

2.1.4.1 Name

Peaton virus NS/3 strain or strain approved as equivalent thereof

2.1.4.2 Properties

When this virus strain is inoculated intravenously in cows, viremia is present, but no clinical symptoms such as fever are observed.

The strain shall be propagated in the following cells in which CPE is confirmed: BHK-21 cells, HmLu-1 cells, HmLu-SC cells, and Vero cells.

2.1.4.3 Master seed virus

2.1.4.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in HmLu cells or cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.

The master seed virus shall not be passaged for purposes other than the production of vaccines. The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.4.4 Working seed virus

2.1.4.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in HmLu cells or cells approved as suitable.

The working seed virus shall be stored frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.4.5 Production seed virus

2.1.4.5.1 Propagation and storage

The production seed virus shall be propagated in HmLu cells or cells approved as suitable.

The production seed virus shall be stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.2 Materials for production

2.2.1 Cell line

HmLu cells or cells approved as suitable shall be used.

2.2.2 Culture medium

The culture medium approved as suitable for production shall be used.

2.2.3 Master cell seeds

2.2.3.1 Preparing, storing, and maximum passage number allowed for obtaining production cell seeds

The master cell seeds shall be propagated in a culture medium in 2.2.2, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master cell seeds shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower.

The master cell seeds shall be tested as specified in 3.2.1.

The master cell seeds shall not be passaged for purposes other than production or testing of vaccines. The master cell seeds shall not be passaged more than 20 times to obtain the production cell seeds.

2.2.4 Working cell seeds

2.2.4.1 Propagation, passage, and storage

The working cell seeds shall be propagated and passaged using culture medium in 2.2.2.

The working cell seeds shall be stored frozen at -70ºC or lower.

The working cell seeds shall be tested as specified in 3.2.2.

2.2.5 Production cell seeds

2.2.5.1 Propagation and storage

The production cell seeds shall be propagated using culture medium in 2.2.2.

The production cell seeds shall not be stored.

2.3 Bulk material

2.3.1 Akabane virus bulk material

2.3.1.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as individual cultured cells. No abnormalities shall be detected prior to the inoculation of the virus.

2.3.1.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.1.1. The filtrate, supernatant after centrifugation, or concentrate of the culture medium collected from individual cultured cells at a time deemed appropriate shall serve as the virus suspension.

The virus suspension shall be tested as specified in 3.3.1 and 3.3.2.1.

2.3.1.3 Inactivation

Virus suspension shall be inactivated by the addition of an inactivator approved as appropriate and shall serve as the inactivated virus suspension.

The inactivated virus suspension shall be tested as specified in 3.4.1.1.

2.3.1.4 Bulk material

The inactivated virus suspension shall be concentrated by a method approved as suitable and shall serve as the bulk material.

The bulk material shall be tested as specified in 3.5.

2.3.2 Kasba virus bulk material

2.3.2.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as individual cultured cells. No abnormalities shall be detected prior to the inoculation of the virus.

2.3.2.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.2.1. The filtrate, supernatant after centrifugation, or concentrate of the culture medium collected from individual cultured cells at a time deemed appropriate shall serve as the virus suspension.

The virus suspension shall be tested as specified in 3.3.1 and 3.3.2.2.

2.3.2.3 Inactivation

Virus suspension shall be inactivated by the addition of an inactivator approved as appropriate and shall serve as the inactivated virus suspension.

The inactivated virus suspension shall be tested as specified in 3.4.1.2.

2.3.2.4 Bulk material

The inactivated virus suspension shall be concentrated by a method approved as suitable and shall serve as the bulk material.

The bulk material shall be tested as specified in 3.5.

2.3.3 Aino virus bulk material

2.3.3.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as individual cultured cells. No abnormalities shall be detected prior to the inoculation of the virus.

2.3.3.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.3.1. The filtrate, supernatant after centrifugation, or concentrate of the culture medium collected from individual cultured cells at a time deemed appropriate shall serve as the virus suspension.

The virus suspension shall be tested as specified in 3.3.1 and 3.3.2.3.

2.3.3.3 Inactivation

Virus suspension shall be inactivated by the addition of an inactivator approved as appropriate and shall serve as the inactivated virus suspension.

The inactivated virus suspension shall be tested as specified in 3.4.1.3.

2.3.3.4 Bulk material

The inactivated virus suspension shall be concentrated by a method approved as suitable and shall serve as the bulk material.

The bulk material shall be tested as specified in 3.5.

2.3.4 Peaton virus bulk material

2.3.4.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as individual cultured cells. No abnormalities shall be detected prior to the inoculation of the virus.

2.3.4.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.4.1. The filtrate, supernatant after centrifugation, or concentrate of the culture medium collected from individual cultured cells at a time deemed appropriate shall serve as the virus suspension.

The virus suspension shall be tested as specified in 3.3.1 and 3.3.2.4.

2.3.4.3 Inactivation

Virus suspension shall be inactivated by the addition of an inactivator approved as appropriate and shall serve as the inactivated virus suspension.

The inactivated virus suspension shall be tested as specified in 3.4.1.4.

2.3.4.4 Bulk material

The inactivated virus suspension shall be concentrated by a method approved as suitable and shall serve as the bulk material.

The bulk material shall be tested as specified in 3.5.

2.4 Final bulk

The Akabane virus bulk material, Kasba virus bulk material, Aino virus bulk material and Peaton virus bulk material shall be adjusted for their antigen levels and mixed, and then mixed with an aluminum gel adjuvant or adjuvant approved as suitable and shall serve as the final bulk.

2.5 Final product

The final bulk shall be dispensed into small containers to serve as the final product.

The final product shall be tested as specified in 3.6.

3 Test methods

3.1 Tests on virus strain used for production

3.1.1 Tests on master seed virus

3.1.1.1 Identification test

The tests given in 1.4.2.3.1.1 in the Seed Lot Specifications shall apply.

3.1.1.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.1.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.1.4 Test for freedom from extraneous viruses

3.1.1.4.1 Common test for freedom from extraneous viruses

The tests given in 1.1, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.2 Test for freedom from specific viruses

3.1.1.4.2.1 General test for freedom from specific viruses

The tests given in 1.1 and 3.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.1, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.2 Tests on working seed virus

3.1.2.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.2.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.3 Tests on production seed virus

3.1.3.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.3.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2 Tests on cell line

3.2.1 Tests on master cell seeds

3.2.1.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.1.1 in the Seed Lot Specifications shall apply.

3.2.1.2 Test for identification of the animal species of the cell

The tests given in 2.1.4.2.1.2 in the Seed Lot Specifications shall apply.

3.2.1.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.1.4 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.1.5 Test for freedom from extraneous viruses

3.2.1.5.1 Common test for freedom from extraneous viruses

The tests given in 1.2, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.5.2 Test for freedom from specific viruses

3.2.1.5.2.1 General test for freedom from specific viruses

The tests given in 1.1 and 3.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.5.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.2, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.6 Test for karyological (chromosomal) characterization

The tests given in 2.1.4.2.1.6 in the Seed Lot Specifications shall apply.

3.2.1.7 Test for freedom from tumorigenicity

The tests given in 2.1.4.2.1.7 in the Seed Lot Specifications shall apply.

3.2.2 Tests on working cell seeds

3.2.2.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.2.1 in the Seed Lot Specifications shall apply.

3.2.2.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.2.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.3 Tests on virus suspension

3.3.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.3.2 Test for virus content

3.3.2.1 Akabane virus

3.3.2.1.1 Materials

3.3.2.1.1.1 Test materials

The test article shall be diluted ten-fold with the virus growth medium approved as suitable, and each level dilution shall serve as the test material.

3.3.2.1.1.2 Cultured cells

HmLu-1 cells shall be used.

3.3.2.1.2 Test procedures

The 0.1 mL portions of the test material shall be inoculated into at least four portions (wells) of cultured cells and allowed to stand for adsorption at 37ºC for 60 minutes. The 1.0 mL portions of the virus growth medium approved as suitable shall be added, cultured at 37ºC for seven days, and observed.

3.3.2.1.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 106.5TCID50 or higher unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

3.3.2.2 Kasba virus

3.3.2.2.1 Materials

3.3.2.2.1.1 Test materials

The test article shall be diluted ten-fold with the virus growth medium approved as suitable, and each level dilution shall serve as the test material.

3.3.2.2.1.2 Cultured cells

Vero-T cells shall be used.

3.3.2.2.2 Test procedures

The 0.1 mL portions of the test material shall be inoculated into at least four portions (wells) of cultured cells and allowed to stand for adsorption at 36ºC for 60 minutes. The 1.0 mL portions of the virus growth medium approved as suitable shall be added, cultured at 37ºC for seven days, and observed.

3.3.2.2.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 107.5TCID50 or higher unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

3.3.2.3 Aino virus

3.3.2.3.1 Materials

3.3.2.3.1.1 Test materials

The test article shall be diluted ten-fold with the virus growth medium approved as suitable, and each level dilution shall serve as the test material.

3.3.2.3.1.2 Cultured cells

HmLu-1 cells shall be used.

3.3.2.3.2 Test procedures

The 0.1 mL portions of the test material shall be inoculated into at least four portions (wells) of cultured cells and allowed for adsorption at 36ºC for 60 minutes. The 1.0 mL portions of the virus growth medium approved as suitable shall be added, cultured at 37ºC for seven days, and observed.

3.3.2.3.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 106.5TCID50 or higher unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

3.3.2.4 Peaton virus

3.3.2.4.1 Materials

3.3.2.4.1.1 Test materials

The test article shall be diluted ten-fold with the virus growth medium approved as suitable, and each level dilution shall serve as the test material.

3.3.2.4.1.2 Cultured cells

HmLu-1 cells shall be used.

3.3.2.4.2 Test procedures

The 0.1 mL portions of the test material shall be inoculated into at least four portions (wells) of cultured cells and allowed to stand for adsorption at 37ºC for 60 minutes. The 1.0 mL portions of the virus growth medium approved as suitable shall be added, cultured at 37ºC for seven days, and observed.

3.3.2.4.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 107.5TCID50 or higher unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

3.4 Tests on inactivated virus suspension

3.4.1 Inactivation test

3.4.1.1 Akabane virus

3.4.1.1.1 Materials

3.4.1.1.1.1 Test materials

A 5 mL portion of the test article shall be dialyzed overnight at 4ºC using at least a 100-fold volume of phosphate-buffered saline to remove the inactivator, and it shall be used as the test material.

3.4.1.1.1.2 Cultured cells

HmLu-1 cells shall be cultured, and monolayer cultures shall be used.

3.4.1.1.2 Test procedures

Whole test material shall be inoculated to the cultured cell sheet of at least 3 cm2 per 1 mL portion, allowed to stand for adsorption at 34ºC for 60 minutes, cultured with culture medium approved as suitable at 37ºC for seven days, and observed.

3.4.1.1.3 Judgment

The cultured cells in which no CPE is observed shall be judged to be negative for active virus.

No active virus shall be observed in the test material.

3.4.1.2 Kasba virus

3.4.1.2.1 Materials

3.4.1.2.1.1 Test materials

A 5 mL portion of the test article shall be dialyzed overnight at 4ºC using at least a 100-fold volume of phosphate-buffered saline to remove the inactivator, and it shall be used as the test material.

3.4.1.2.1.2 Cultured cells

Vero-T cells shall be cultured, and monolayer cultures shall be used.

3.4.1.2.2 Test procedures

Whole test material shall be inoculated to the cultured cell sheet of at least 3 cm2 per 1 mL portion, allowed to stand for adsorption at 34ºC for 60 minutes, cultured with culture medium approved as suitable at 34 – 36ºC for five days, and then the cells shall be passaged to the next generation. After the cell layers are formed, culture media shall be sampled. The culture medium approved as suitable shall be added, cultured at 34ºC to 36ºC for five days, passaged to subsequent generations, and then cultured in the same manner as the second generation and observed.

3.4.1.2.3 Judgment

The cultured cells in which no CPE is observed shall be judged to be negative for active virus.

No active virus shall be observed in the test material.

3.4.1.3 Aino virus

3.4.1.3.1 Materials

3.4.1.3.1.1 Test materials

A 5 mL portion of the test article shall be dialyzed overnight at 4ºC using at least a 100-fold volume of phosphate-buffered saline to remove the inactivator, and it shall be used as the test material.

3.4.1.3.1.2 Cultured cells

HmLu-1 cells shall be cultured, and monolayer cultures shall be used.

3.4.1.3.2 Test procedures

Whole test material shall be inoculated to the cultured cell sheet of at least 3 cm2 per 1 mL portion, allowed to stand for adsorption at 34ºC for 60 minutes, cultured with culture medium approved as suitable at 34ºC to 36ºC for seven days, and observed.

3.4.1.3.3 Judgment

The cultured cells in which no CPE is observed shall be judged to be negative for active virus.

No active virus shall be observed in the test material.

3.4.1.4 Peaton virus

3.4.1.4.1 Materials

3.4.1.4.1.1 Test materials

A 5 mL portion of the test article shall be dialyzed overnight at 4ºC using at least a 100-fold volume of phosphate-buffered saline to remove the inactivator, and it shall be used as the test material.

3.4.1.4.1.2 Cultured cells

HmLu-1 cells shall be cultured, and monolayer cultures shall be used.

3.4.1.4.2 Test procedures

Whole test sample shall be inoculated to the cultured cell sheet of at least 3 cm2 per 1 mL portion, allowed to stand for adsorption at 34ºC for 60 minutes, cultured with culture medium approved as suitable at 34ºC to 36ºC for seven days, and observed.

3.4.1.4.3 Judgment

The cultured cells in which no CPE is observed shall be judged to be negative for active virus.

No active virus shall be observed in the test article.

3.5 Tests on bulk material

3.5.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.6 Tests on final product

3.6.1 Properties test

When the test is performed as specified in the Properties Test of the General Tests, the final product shall be a homogeneous suspension with a specific color, and no foreign matter and foreign odor shall be observed. The property of the product per small container shall be uniform.

3.6.2 Test for pH

When the test is performed as specified in the Test for pH of the General Tests, the pH shall have an intrinsic value.

3.6.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.6.4 Test for formalin content

For products inactivated with formalin when the test given in the Test for Formalin Content of the General Tests is applied, the formalin content shall be no higher than 0.05 vol%.

3.6.5 Test for aluminum content

When the test is performed as specified in the Test for Aluminum Content of the General Tests, the content of aluminum shall have a specific value. When the Minister of Agriculture, Forestry and Fisheries specifies the test procedure, follow it.

3.6.6 Test for freedom from abnormal toxicity

The test given in the Test for Freedom from Abnormal Toxicity of the General Tests shall apply.

3.6.7 Potency test

3.6.7.1 Materials

3.6.7.1.1 Injection materials

The test samples shall be used as injection materials.

3.6.7.1.2 Test animals

Guinea pigs weighing approximately 350 g shall be used.

3.6.7.1.3 Virus for neutralization test

3.6.7.1.3.1 Akabane virus

Akabane virus JaGAr 39 strain propagated in HmLu cells or strain approved as equivalent thereof shall be used.

3.6.7.1.3.2 Kasba virus

Kasba virus K-47 strain propagated in BHK-21 cells or strain approved as equivalent thereof shall be used.

3.6.7.1.3.3 Aino virus

Aino virus JaNAr 28 strain propagated in HmLu cells or strain approved as equivalent thereof shall be used.

3.6.7.1.3.4 Peaton virus

Peaton virus NS/3 strain propagated in HmLu cells or strain approved as equivalent thereof shall be used.

3.6.7.1.4 Cultured cells

HmLu-1 cells and Vero-T cells shall be used.

3.6.7.2 Test procedures

A 0.5 mL each of the injection materials shall be injected intramuscularly twice at intervals of three weeks in five test animals, and serum shall be obtained from individual animals 10 days after the second injection to conduct a neutralization test.

The test serum shall be heat-inactivated, and then diluted with culture medium approved as suitable in two-fold serial dilutions. Mix equal quantities of each diluted serum and virus suspension for neutralization test containing approximately 200 TCID50 in 0.1 mL and neutralize at 37ºC for 60 minutes. For the Kasba virus, the mixture shall be neutralized for 90 minutes. The mixtures obtained shall be inoculated at a dose of 0.1 mL into four portions (wells) of cultured HmLu-1 cells for the Akabane virus, Aino virus, and Peaton virus, respectively, and into four portions (wells) of cultured Vero-T cells for the Kasba virus, respectively, and allowed to stand for adsorption at 37ºC for 60 minutes. Then, culture media approved as suitable shall be added at a dose of 1.0 mL and cultured at 37ºC for seven days for the Akabane virus, Kasba virus, and Peaton virus and at 34ºC to 36ºC for seven days for the Aino virus.

3.6.7.3 Judgment

The maximum dilution factor of serum at which CPE is observed in at least two portions of cultured cells shall be defined as the neutralizing antibody titer.

A 16-fold or greater increase in the neutralizing antibody titer against the Akabane virus and Peaton virus, a 32-fold or greater increase in the neutralizing antibody titer against the Kasba virus and an 8-fold or greater increase in the neutralizing antibody titer against the Aino virus shall be regarded as positive for neutralizing antibodies.

The neutralizing antibody positive rates in test animals shall be 80% or higher against each virus.

4 Storage and expiry date

The expiry date shall be two years and three months after the manufacturing unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

**Infection Bovine Rhinotracheitis, Bovine Viral Diarrhea (bivalent), Bovine Parainfluenza, Bovine Respiratory Syncytial Virus**

**Infection Vaccine (adjuvant), Inactivated, Seed**

1 Definition

This Vaccine, Inactivated, Seed is made by mixing the inactivated virus suspensions obtained by propagating Infections bovine rhinotracheitis virus, Bovine viral diarrhea virus type 1, Bovine viral diarrhea virus type 2, Bovine parainfluenza type 3 virus, and Bovine respiratory syncytial virus that meet the Seed Lot Specifications in cell lines that meet the said specifications, respectively, and by adding an adjuvant.

2 Production methods

2.1 Virus strain used for production

2.1.1 Infectious bovine rhinotracheitis virus

2.1.1.1 Name

Infectious bovine rhinotracheitis virus No. 758-KB strain or strain approved as equivalent thereof

2.1.1.2 Properties

This virus strain shows the pathogenic effects on cows, such as fever, respiratory symptoms, and leukopenia.

The strain shall be propagated in cultured cells derived from bovine and cultured cells derived from swine in which CPE is confirmed.

2.1.1.3 Master seed virus

2.1.1.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in MDBK-NSC cells or cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.1.

The master seed virus shall not be passaged for purposes other than the production of vaccines. The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.1.4 Working seed virus

2.1.1.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in MDBK-NSC cells or cells approved as suitable.

The working seed virus shall be stored frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.1.5 Production seed virus

2.1.1.5.1 Propagation and storage

The production seed virus shall be propagated in MDBK-NSC cells or cells approved as suitable.

The production seed virus shall be stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.1.2 Bovine viral diarrhea virus type 1

2.1.2.1 Name

Bovine viral diarrhea virus type 1 Nose-KB strain or strain approved as equivalent thereof

2.1.2.2 Properties

Inoculation of this virus strain into the nasal cavity of cows causes respiratory symptoms, fever viremia, and leukopenia.

The strain shall be propagated in cultured cells derived from bovine in which CPE is confirmed, and if co-infected with cells infected with the non-cytopathogenic strain, CPE is inhibited.

2.1.2.3 Master seed virus

2.1.2.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in MDBK-NSC cells or cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.2.

The master seed virus shall not be passaged for purposes other than the production of vaccines. The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.2.4 Working seed virus

2.1.2.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in MDBK-NSC cells or cells approved as suitable.

The working seed virus shall be stored frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.2.5 Production seed virus

2.1.2.5.1 Propagation and storage

The production seed virus shall be propagated in MDBK-NSC cells or cells approved as suitable.

The production seed virus shall be stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.1.3 Bovine viral diarrhea virus type 2

2.1.3.1 Name

Bovine viral diarrhea virus type 2 KZ-91-KB strain or strain approved as equivalent thereof

2.1.3.2 Properties

Inoculation of this virus strain into the nasal cavity of calves causes respiratory symptoms, fever, viremia, and leukopenia.

The strain shall be propagated in cultured cells derived from bovine in which CPE is confirmed, and if co-infected with cells infected with the non-cytopathogenic strain, CPE is inhibited.

2.1.3.3 Master seed virus

2.1.3.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in MDBK-NSC cells or cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.3.

The master seed virus shall not be passaged for purposes other than the production of vaccines. The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.3.4 Working seed virus

2.1.3.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in MDBK-NSC cells or cells approved as suitable.

The working seed virus shall be stored frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.3.5 Production seed virus

2.1.3.5.1 Propagation and storage

The production seed virus shall be propagated in MDBK-NSC cells or cells approved as suitable.

The production seed virus shall be stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.1.4 Bovine parainfluenza type 3 virus

2.1.4.1 Name

Bovine parainfluenza type 3 virus BN1-1-KB strain or strain approved as equivalent thereof

2.1.4.2 Properties

This virus stain shows the pathogenic effects on cows, such as fever and respiratory symptoms.

The strain shall be propagated in cultured cells derived from bovine and cell lines derived from African green monkey kidneys (hereinafter referred to as “Vero cells” in this paragraph) in which CPE is confirmed.

2.1.4.3 Master seed virus

2.1.4.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in MDBK-NSC cells or cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.4.

The master seed virus shall not be passaged for purposes other than the production of vaccines.

The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.4.4 Working seed virus

2.1.4.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in MDBK-NSC cells or cells approved as suitable.

The working seed virus shall be stored frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.4.5 Production seed virus

2.1.4.5.1 Propagation and storage

The production seed virus shall be propagated in MDBK-NSC cells or cells approved as suitable.

The production seed virus shall be stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.1.5 Bovine respiratory syncytial virus

2.1.5.1 Name

Bovine respiratory syncytial virus rs-52-KB strain or strain approved as equivalent thereof

2.1.5.2 Properties

Inoculation of this virus stain on cows cause no pathogenic effects.

The strain shall be propagated in the following cells in which CPE is confirmed: cells derived from bovine, cells derived from hamster lung and Vero cells.

2.1.5.3 Master seed virus

2.1.5.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in HmLu-SC cells or cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.5.

The master seed virus shall not be passaged for purposes other than the production of vaccines. The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.5.4 Working seed virus

2.1.5.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in HmLu-SC cells or cells approved as suitable.

The working seed virus shall be stored frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.5.5 Production seed virus

2.1.5.5.1 Propagation and storage

Production seed virus shall be propagated in HmLu-SC cells or cells approved as suitable.

The production seed virus shall be stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.2 Materials for production

2.2.1 Infectious bovine rhinotracheitis virus, Bovine viral diarrhea virus type 1, Bovine viral diarrhea virus type 2, and Bovine parainfluenza type 3 virus

2.2.1.1 Cultured cells

MDBK-NSC cells or cell lines approved as suitable shall be used.

2.2.1.2 Culture medium

The culture medium approved as suitable for production shall be used.

2.2.1.3 Master cell seeds

2.2.1.3.1 Preparing, storing, and maximum passage number allowed for obtaining production cell seeds

The master cell seeds shall be propagated in a culture medium in 2.2.1.2, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master cell seeds shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower.

The master cell seeds shall be tested as specified in 3.2.1.1.

The master cell seeds shall not be passaged for purposes other than production or testing of vaccines.

The master cell seeds shall not be passaged more than 20 times to obtain the production cell seeds.

2.2.1.4 Working cell seeds

2.2.1.4.1 Propagation, passage, and storage

The working cell seeds shall be propagated and passaged using culture medium in 2.2.1.2.

The working cell seeds shall be stored frozen at -70ºC or lower.

The working cell seeds shall be tested as specified in 3.2.2.1.

2.2.1.5 Production cell seeds

2.2.1.5.1 Propagation and storage

The production cell seeds shall be propagated using culture medium in 2.2.1.2.

The production cell seeds shall be stored frozen at -70°C or lower when it needs to be stored.

The production cell seeds shall be tested as specified in 3.2.3.1 if they need to be stored.

2.2.2 Bovine respiratory syncytial virus

2.2.2.1 Cultured cells

HmLu-SC cells or cell lines approved as suitable shall be used.

2.2.2.2 Culture medium

The culture medium approved as suitable for production shall be used.

2.2.2.3 Master cell seeds

2.2.2.3.1 Preparing, storing, and maximum passage number allowed for obtaining production cell

seeds

The master cell seeds shall be propagated in a culture medium in 2.2.2.2, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master cell seeds shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower.

The master cell seeds shall be tested as specified in 3.2.1.2.

The master cell seeds shall not be passaged for purposes other than production or testing of vaccines. The master cell seeds shall not be passaged more than 20 times to obtain the production cell seeds.

2.2.2.4 Working cell seeds

2.2.2.4.1 Propagation, passage, and storage

The working cell seeds shall be propagated and passaged using culture medium in 2.2.2.2.

The working cell seeds shall be stored frozen at -70ºC or lower.

The working cell seeds shall be tested as specified in 3.2.2.2.

2.2.2.5 Production cell seeds

2.2.2.5.1 Propagation and storage

The production cell seeds shall be propagated using culture medium in 2.2.2.2.

The production cell seeds shall be stored frozen at -70°C or lower when it needs to be stored.

The production cell seeds shall be tested as specified in 3.2.3.2 if they need to be stored.

2.3 Bulk material

2.3.1 Infectious bovine rhinotracheitis virus

2.3.1.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as individual cultured cells. No abnormalities shall be detected prior to the inoculation of the virus.

2.3.1.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.1.1. The filtrate, supernatant after centrifugation, or concentrate of the culture medium collected from individual cultured cells at a time deemed appropriate shall serve as the virus suspension.

The virus suspension shall be tested as specified in 3.3.1 and 3.3.2.1.

2.3.1.3 Inactivation

The virus suspension inactivated by treating it with a method approved as suitable shall be used as the bulk material.

The bulk material shall be tested as specified in 3.4.1 and 3.4.2.1.

2.3.2 Bovine viral diarrhea virus type 1

2.3.2.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as individual cultured cells. No abnormalities shall be detected in production cell seeds prior to the inoculation of the virus.

2.3.2.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.2.1. The filtrate, supernatant after centrifugation, or concentrate of the culture medium collected from individual cultured cells at a time deemed appropriate shall serve as the virus suspension.

The virus suspension shall be tested as specified in 3.3.1 and 3.3.2.2.

2.3.2.3 Inactivation

The virus suspension inactivated by treating it with a method approved as suitable shall be used as the bulk material.

The bulk material shall be tested as specified in 3.4.1 and 3.4.2.1.

2.3.3 Bovine viral diarrhea virus type 2

2.3.3.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as individual cultured cells. No abnormalities shall be detected prior to the inoculation of the virus.

2.3.3.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.3.1. The filtrate, supernatant after centrifugation, or concentrate of the culture medium collected from individual cultured cells at a time deemed appropriate shall serve as the virus suspension.

The virus suspension shall be tested as specified in 3.3.1 and 3.3.2.3.

2.3.3.3 Inactivation

The virus suspension inactivated by treating it with a method approved as suitable shall be used as the bulk material.

The bulk material shall be tested as specified in 3.4.1 and 3.4.2.1.

2.3.4 Bovine parainfluenza type 3 virus

2.3.4.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as individual cultured cells. No abnormalities shall be detected prior to the inoculation of the virus.

2.3.4.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.4.1. The filtrate, supernatant after centrifugation, or concentrate of the culture medium collected from individual cultured cells at a time deemed appropriate shall serve as the virus suspension.

The virus suspension shall be tested as specified in 3.3.1 and 3.3.2.4.

2.3.4.3 Inactivation

The virus suspension inactivated by treating it with a method approved as suitable shall be used as the bulk material.

The bulk material shall be tested as specified in 3.4.1 and 3.4.2.1.

2.3.5 Bovine respiratory syncytial virus

2.3.5.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as individual cultured cells. No abnormalities shall be detected in production cell seeds prior to the inoculation of the virus.

2.3.5.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.5.1. The filtrate, supernatant after centrifugation, or concentrate of the culture medium collected from individual cultured cells at a time deemed appropriate shall serve as the virus suspension.

The virus suspension shall be tested as specified in 3.3.1 and 3.3.2.5.

2.3.5.3 Inactivation

The virus suspension inactivated by treating it with a method approved as suitable shall serve as the bulk material.

The bulk material shall be tested as specified in 3.4.1 and 3.4.2.2.

2.4 Final bulk

Mix Infectious bovine rhinotracheitis virus bulk material, Bovine viral diarrhea virus type 1 bulk material, Bovine viral diarrhea virus type 2 bulk material, Bovine parainfluenza type 3 virus bulk material, and Bovine respiratory syncytial virus bulk, add adjuvants and preservatives approved as suitable, and then adjust the concentration of the mixture to serve as the final bulk.

2.5 Final product

The final bulk shall be dispensed into small containers to serve as the final product.

The final product shall be tested as specified in 3.5.

3 Test methods

3.1 Tests on virus strain used for production

3.1.1 Tests on master seed virus

3.1.1.1 Infectious bovine rhinotracheitis virus

3.1.1.1.1 Identification test

The tests given in 1.4.2.3.1.1.2 in the Seed Lot Specifications shall apply.

3.1.1.1.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.1.1.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.1.1.4 Test for freedom from extraneous viruses

3.1.1.1.4.1 Common test for freedom from extraneous viruses

The tests given in 1.1, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.1.4.2 Test for freedom from specific viruses

3.1.1.1.4.2.1 General test for freedom from specific viruses

For the Bovine respiratory syncytial virus and Bluetongue virus, the tests given in 1.1 and 3.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.1.4.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.1, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.2 Bovine viral diarrhea virus type 1

3.1.1.2.1 Identification test

The tests given in 1.4.2.3.1.1.2 in the Seed Lot Specifications shall apply.

3.1.1.2.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.1.2.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.1.2.4 Test for freedom from extraneous viruses

3.1.1.2.4.1 Common test for freedom from extraneous viruses

The tests given in 1.1, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.2.4.2 Test for freedom from specific viruses

3.1.1.2.4.2.1 General test for freedom from specific viruses

For the Bovine respiratory syncytial virus and Bluetongue virus, the tests given in 3.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.2.4.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.1, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.3 Bovine viral diarrhea virus type 2

3.1.1.3.1 Identification test

The tests given in 1.4.2.3.1.1.2 in the Seed Lot Specifications shall apply.

3.1.1.3.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.1.3.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.1.3.4 Test for freedom from extraneous viruses

3.1.1.3.4.1 Common test for freedom from extraneous viruses

The tests given in 1.1, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.3.4.2 Test for freedom from specific viruses

3.1.1.3.4.2.1 General test for freedom from specific viruses

For the Bovine respiratory syncytial virus and Bluetongue virus, the tests given in 3.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.3.4.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.1, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4 Bovine parainfluenza type 3 virus

3.1.1.4.1 Identification test

The tests given in 1.4.2.3.1.1.2 in the Seed Lot Specifications shall apply.

3.1.1.4.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.1.4.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.1.4.4 Test for freedom from extraneous viruses

3.1.1.4.4.1 Common test for freedom from extraneous viruses

The tests given in 1.1, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.4.2 Test for freedom from specific viruses

3.1.1.4.4.2.1 General test for freedom from specific viruses

For the Bovine respiratory syncytial virus and Bluetongue virus, the tests given in 1.1 and 3.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.4.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.1, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.5 Bovine respiratory syncytial virus

3.1.1.5.1 Identification test

The tests given in 1.4.2.3.1.1.2 in the Seed Lot Specifications shall apply.

3.1.1.5.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.1.5.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.1.5.4 Test for freedom from extraneous viruses

3.1.1.5.4.1 Common test for freedom from extraneous viruses

The tests given in 1.1, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.5.4.2 Test for freedom from specific viruses

3.1.1.5.4.2.1 General test for freedom from specific viruses

For the Lymphocytic choriomeningitis virus and Bluetongue virus, the tests given in 1.1 and 3.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.5.4.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.1, 3.2.5, 3.2.7, 3.2.8 and 2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.2 Tests on working seed virus

3.1.2.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.2.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.3 Tests on production seed virus

3.1.3.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.3.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2 Tests on cultured cells

3.2.1 Tests on master cell seeds

3.2.1.1 MDBK-NSC cells

3.2.1.1.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.1.1 in the Seed Lot Specifications shall apply.

3.2.1.1.2 Test for identification of the animal species of the cell

The tests given in 2.1.4.2.1.2 in the Seed Lot Specifications shall apply.

3.2.1.1.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.1.1.4 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.1.1.5 Test for freedom from extraneous viruses

3.2.1.1.5.1 Common test for freedom from extraneous viruses

The tests given in 1.2, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.1.5.2 Test for freedom from specific viruses

3.2.1.1.5.2.1 General test for freedom from specific viruses

For the Bovine respiratory syncytial virus and Bluetongue virus, the tests given in 1.2 and 3.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.1.5.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.2, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.1.6 Test for karyological (chromosomal) characterization

The tests given in 2.1.4.2.1.6 in the Seed Lot Specifications shall apply.

3.2.1.1.7 Test for freedom from tumorigenicity

The tests given in 2.1.4.2.1.7 in the Seed Lot Specifications shall apply.

3.2.1.2 HmLu-SC cells

3.2.1.2.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.1.1 in the Seed Lot Specifications shall apply.

3.2.1.2.2 Test for identification of the animal species of the cell

The tests given in 2.1.4.2.1.2 in the Seed Lot Specifications shall apply.

3.2.1.2.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.1.2.4 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.1.2.5 Test for freedom from extraneous viruses

3.2.1.2.5.1 Common test for freedom from extraneous viruses

The tests given in 1.2, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.2.5.2 Test for freedom from specific viruses

3.2.1.2.5.2.1 General test for freedom from specific viruses

For the Bovine respiratory syncytial virus, Bluetongue virus, and Lymphocytic choriomeningitis virus, the tests given in 1.2 and 3.1 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.2.5.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Rotavirus, Bovine leukemia virus, Japanese encephalitis virus, and Rabies virus, the tests given in 1.2, 3.2.5, 3.2.7, 3.2.8 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.2.6 Test for karyological (chromosomal) characterization

The tests given in 2.1.4.2.1.6 in the Seed Lot Specifications shall apply.

3.2.1.2.7 Test for freedom from tumorigenicity

The tests given in 2.1.4.2.1.7 in the Seed Lot Specifications shall apply.

3.2.2 Tests on working cell seeds

3.2.2.1 MDBK-NSC cells

3.2.2.1.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.2.1 in the Seed Lot Specifications shall apply.

3.2.2.1.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.2.1.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.2.2 HmLu-SC cells

3.2.2.2.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.2.1 in the Seed Lot Specifications shall apply.

3.2.2.2.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.2.2.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.3 Tests on production cell seeds

3.2.3.1 MDBK-NSC cells

3.2.3.1.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.3.1 in the Seed Lot Specifications shall apply.

3.2.3.1.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.3.1.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.3.2 HmLu-SC cells

3.2.3.2.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.3.1 in the Seed Lot Specifications shall apply.

3.2.3.2.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.3.2.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.3 Tests on virus suspension

3.3.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.3.2 Test for virus content

3.3.2.1 Infectious bovine rhinotracheitis virus

3.3.2.1.1 Materials

3.3.2.1.1.1 Test materials

The test article shall be diluted ten-fold with cell growth medium (Note 1) and each level dilution shall serve as the test material.

3.3.2.1.1.2 Cultured cells

Cell line suspension derived from bovine kidneys (hereinafter referred to as “MDBK cells” in this paragraph) shall be used.

3.3.2.1.2 Test procedures

The 100 μL portions of the test material shall be inoculated into 100 μL portions of cultured cells dispensed into at least 4 wells in a 96-well microplate, cultured in an air containing 5 vol% carbon dioxide at 37ºC for seven days, and observed.

3.3.2.1.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 108.0TCID50 or higher.

3.3.2.2 Bovine viral diarrhea virus type 1

3.3.2.2.1 Materials

3.3.2.2.1.1 Test materials

The test article shall be diluted ten-fold with cell growth medium and each level dilution shall serve as the test material.

3.3.2.2.1.2 Cultured cells

Bovine testis cell suspension shall be used.

3.3.2.2.2 Test procedures

The 100 μL portions of the test sample shall be inoculated into 100 μL portions of cultured cells dispensed into at least 4 wells in a 96-well microplate, cultured in an air containing 5 vol% carbon dioxide at 37ºC for seven days, and observed.

3.3.2.2.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 107.6TCID50 or higher.

3.3.2.3 Bovine viral diarrhea virus type 2

3.3.2.3.1 Materials

3.3.2.3.1.1 Test materials

The test article shall be diluted ten-fold with cell growth medium and each level dilution shall serve as the test material.

3.3.2.3.1.2 Cultured cells

Bovine testis cell suspension shall be used.

3.3.2.3.2 Test procedures

The 100 μL portions of the test sample shall be inoculated into 100 μL portions of cultured cells dispensed into at least 4 wells in a 96-well microplate, cultured in an air containing 5 vol% carbon dioxide at 37ºC for seven days, and observed.

3.3.2.3.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 106.4TCID50 or higher.

3.3.2.4 Bovine parainfluenza type 3 virus

3.3.2.4.1 Materials

3.3.2.4.1.1 Test materials

The test article shall be diluted ten-fold with cell growth medium and each level dilution shall serve as the test material.

3.3.2.4.1.2 Cultured cells

MDBK cell suspension shall be used.

3.3.2.4.2 Test procedures

The 100 μL portions of the test sample shall be inoculated into 100 μL portions of cultured cells dispensed into at least 4 wells in a 96-well microplate, cultured in an air containing 5 vol% carbon dioxide at 37ºC for seven days, and observed.

3.3.2.4.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 107.9TCID50 or higher.

3.3.2.5 Bovine respiratory syncytial virus

3.3.2.5.1 Materials

3.3.2.5.1.1 Test materials

The test article shall be diluted ten-fold with cell growth medium and each level dilution shall serve as the test material.

3.3.2.5.1.2 Cultured cells

Vero cell suspension shall be used.

3.3.2.5.2 Test procedures

The 100 μL portions of the test sample shall be inoculated into 100 μL portions of cultured cells dispensed into at least 4 wells in a 96-well microplate, cultured in an air containing 5 vol% carbon dioxide at 34ºC for seven days, and observed.

3.3.2.5.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 106.6TCID50 or higher.

3.4 Tests on bulk material

3.4.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.4.2 Inactivation test

3.4.2.1 Infectious bovine rhinotracheitis virus, Bovine viral diarrhea virus type 1, Bovine viral diarrhea virus type 2 and Bovine parainfluenza type 3 virus

3.4.2.1.1 Materials

3.4.2.1.1.1 Test materials

The test article shall be dialyzed overnight at 4ºC using at least a 100-fold volume of phosphate-buffered saline to remove the inactivator, and it shall be used as the test material.

3.4.2.1.1.2 Cultured cells

MDBK cells shall be cultured for one to three days, and monolayer cultures shall be used.

3.4.2.1.2 Test procedures

A 1 mL portion of the test material shall be inoculated to the cultured cell sheet of at least 3 cm2, allowed to adsorb for 90 minutes at 37ºC, cultured with the virus growth medium (Note 2) at 37ºC for seven days, and observed.

3.4.2.1.3 Judgment

The cultured cells in which no CPE is observed shall be judged to be negative for active virus.

No active virus shall be observed in the test materials.

3.4.2.2 Bovine respiratory syncytial virus

3.4.2.2.1 Materials

3.4.2.2.1.1 Test materials

The test article shall be dialyzed overnight at 4ºC using at least a 100-fold volume of phosphate-buffered saline to remove the inactivator, and it shall be used as the test material.

3.4.2.2.1.2 Cultured cells

Vero cells shall be cultured for one to three days, and monolayer cultures shall be used.

3.4.2.2.2 Test procedures

A 1 mL portion of the test sample shall be inoculated to the cultured cell sheet of at least 3 cm2 or more, allowed to adsorb for 90 minutes at 37ºC, cultured with the virus growth medium at 34ºC for seven days, and observed.

3.4.2.2.3 Judgment

The cultured cells in which no CPE is observed shall be judged to be negative for active virus.

No active virus shall be observed in the test materials.

3.5 Tests on final product

3.5.1 Properties test

When the test is performed as specified in the Properties Test of the General Tests, the final product shall be a homogeneous suspension with a specific color, and no foreign matter and foreign odor shall be observed. The property of the product per small container shall be uniform.

3.5.2 Test for pH

When the test is performed as specified in the Test for pH of the General Tests, the pH shall have an intrinsic value.

3.5.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.5.4 Test for formalin content

When the test given in the Test for Formalin Content of the General Tests is applied, the formalin content shall be no higher than 0.08 vol%.

3.5.5 Test for aluminum content

When the test is performed as specified in the Test for Aluminum Content of the General Tests, the content of aluminum shall be 1.35–1.65 mg in 1 mL.

3.5.6 Toxicity limit test

The test given in the Toxicity Limit Test 2 of the General Tests shall apply. However, verify by a t-test of population mean differences assuming equal variances (unpaired, two-tailed, 5% level of significance) that the body weight on the third day after injected at a dose of 1 mL is equal to or greater than that before injected.

3.5.7 Potency test

3.5.7.1 Potency test on infectious bovine rhinotracheitis

3.5.7.1.1 Materials

3.5.7.1.1.1 Injection materials

The test samples shall be used as injection materials.

3.5.7.1.1.2 Test animals

Guinea pigs weighing approximately 300 g shall be used.

3.5.7.1.1.3 Virus for neutralization test

Infectious bovine rhinotracheitis virus No. 758 strain or strain approved as suitable shall be used.

3.5.7.1.1.4 Cultured cells

MDBK cell suspension shall be used.

3.5.7.1.2 Test procedures

Three mL each of the injection materials shall be injected twice at intervals of two weeks in five test animals. Each test material shall be injected intramuscularly at a volume of 1 mL into two sites and subcutaneously at a volume of 1 mL into one site. The serum shall be obtained from individual animals 10 days after the second injection to conduct a neutralization test.

The serum shall be heat-inactivated, and then shall be diluted two-fold with the virus growth medium. Mix equal quantities of each diluted serum and virus for neutralization test containing approximately 200 TCID50 in 25 μL and neutralize at 37ºC for 18 hours. The 25 μL portions of the mixed solution shall be inoculated into 4 wells in a 96-well microplate, the 0.1 mL portions of the cultured cells shall be added, and cultured in an air containing 5 vol% carbon dioxide at 37ºC for seven days, and observed.

3.5.7.1.3 Judgment

The maximum dilution factor of serum at which inhibition of CPE is observed in at least two wells of cultured cells shall be defined as the neutralizing antibody titer.

When a 128-fold or greater increase in the neutralizing antibody titer is regarded as positive, the neutralizing antibody positive rate in test animals shall be 80% or higher.

3.5.7.2 Potency test on bovine viral diarrhea

3.5.7.2.1 Materials

3.5.7.2.1.1 Injection materials

The test samples shall be used as injection materials.

3.5.7.2.1.2 Test animals

Rats weighing approximately 100 g shall be used.

3.5.7.2.1.3 Virus for neutralization test

3.5.7.2.1.3.1 Bovine viral diarrhea virus type 1

Bovine viral diarrhea virus type 1 Nose strain or strain approved as suitable shall be used.

3.5.7.2.1.3.2 Bovine viral diarrhea virus type 2

Bovine viral diarrhea virus type 2 KZ-91-cp strain or strain approved as suitable shall be used.

3.5.7.2.1.4 Cultured cells

MDBK cell suspension shall be used.

3.5.7.2.2 Test procedures

Two mL each of the injection materials shall be injected intramuscularly at a volume of 1 mL each in thighs of both hindlimbs in five test animals. Serum shall be obtained from individual animals 21 days after the injection to conduct a neutralization test.

The serum shall be heat-inactivated, and then shall be diluted two-fold with the virus growth medium. Mix equal quantities of each diluted serum and virus for neutralization test containing approximately 200 TCID50 in 25 μL and neutralize at 37ºC for 60 minutes. The 25 μL portions of the mixed solution shall be inoculated into 4 wells in a 96-well microplate, the 0.1 mL portions of the cultured cells shall be added, and cultured in an air containing 5 vol% carbon dioxide at 37ºC for seven days, and observed.

3.5.7.2.3 Judgment

The maximum dilution factor of serum at which inhibition of CPE is observed in at least two wells of cultured cells shall be defined as the neutralizing antibody titer.

When a two-fold or greater increase in the neutralizing antibody titer is regarded as positive, the neutralizing antibody positive rates in test animals shall be 80% or higher against bovine viral diarrhea virus type 1 and bovine viral diarrhea virus type 2, respectively.

3.5.7.3 Potency test on bovine parainfluenza

3.5.7.3.1 Materials

3.5.7.3.1.1 Injection materials

The injection materials specified in 3.5.7.1.1.1 shall be used.

3.5.7.3.1.2 Test animals

The test animals specified in 3.5.7.1.1.2 shall be used.

3.5.7.3.1.3 Virus for neutralization test

Bovine parainfluenza type 3 virus BN1-1 strain or strain approved as suitable shall be used.

3.5.7.3.1.4 Cultured cells

Vero cell suspension shall be used.

3.5.7.3.2 Test procedures

The test method given in 3.5.7.1.2 shall apply.

However, the serum to be tested shall be neutralized at 37ºC for 60 minutes.

3.5.7.3.3 Judgment

The maximum dilution factor of serum at which inhibition of CPE is observed in at least two wells of cultured cells shall be defined as the neutralizing antibody titer.

When an at least four-fold increase in the neutralizing antibody titer is regarded as positive, the neutralizing antibody positive rate in test animals shall be 80% or higher.

3.5.7.4 Potency on bovine respiratory syncytial virus infection

3.5.7.4.1 Materials

3.5.7.4.1.1 Injection materials

The injection materials specified in 3.5.7.1.1.1 shall be used.

3.5.7.4.1.2 Test animals

The test animals specified in 3.5.7.1.1.2 shall be used.

3.5.7.4.1.3 Virus for neutralization test

Bovine respiratory syncytial virus NMK7 strain or strain approved as suitable shall be used.

3.5.7.4.1.4 Cultured cells

Vero cells which are grown as monolayers in a 96-well microplate shall be used.

3.5.7.4.2 Test procedures

A neutralization test shall be performed on the serum obtained in 3.5.7.1.2.

The serum shall be heat-inactivated, and then shall be diluted two-fold with the virus growth medium. Equal quantities of each diluted serum and virus for neutralization test containing approximately 200 TCID50 in 25 μL shall be mixed and neutralized at 37ºC for 60 minutes. Removing the culture medium of Vero cells, the 25 μL portions of the mixture shall be added into 4 wells, respectively, and adsorbed in an air containing 5 vol% carbon dioxides at 37ºC for 60 minutes. After the adsorption, the 0.1 mL portions of the virus growth medium shall be added, cultured in an air containing 5 vol% carbon dioxides at 34ºC for seven days, and observed.

3.5.7.4.3 Judgment

The maximum dilution factor of serum at which inhibition of CPE is observed in at least two wells of cultured cells shall be defined as the neutralizing antibody titer.

When a two-fold or greater increase in the neutralizing antibody titer is regarded as positive, the neutralizing antibody positive rate in test animals shall be 80% or higher.

4 Storage and expiry date

The expiry date shall be one year and six months after the manufacturing unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

Note 1 Cell growth medium

1,000 mL consists of the following:

|  |  |
| --- | --- |
| Tryptose phosphate broth | 2.95 g |
| Fetal bovine serum | 50–100 mL |
| Eagle’s MEM | Residual quantity |

The pH shall be adjusted to 7.2–7.6 with sodium hydrogen carbonate.

Fetal bovine serum used shall be suitable for cell maintenance and virus growth.

Minimal amounts of antibiotics may be added.

Note 2 Virus growth medium

1,000 mL consists of the following:

|  |  |
| --- | --- |
| Tryptose phosphate broth | 2.95 g |
| Fetal bovine serum | 20–100 mL |
| Eagle’s MEM | Residual quantity |

The pH shall be adjusted to 7.2–7.6 with sodium hydrogen carbonate.

Fetal bovine serum used shall be suitable for cell maintenance and virus growth.

Minimal amounts of antibiotics may be added.

**Equine Influenza Vaccine, Inactivated**

1 Definition

Equine Influenza Vaccine, Inactivated is an inactivated vaccine produced by the purification and concentration of the virus suspension obtained by propagating Equine influenza viruses in embryonated chicken eggs.

2 Production methods

2.1 Virus strain used for production

2.1.1 Name

Strains of Equine influenza A virus specified separately

2.1.2 Properties

The virus strains shall be propagated in the allantoic cavities of embryonated chicken eggs, and it shall agglutinate chicken red blood cells.

2.1.3 Passage and storage

The original strain and seed virus shall be propagated in the embryonated chicken eggs.

The original strain and seed virus shall not be passaged more than five times and three times, respectively.

This does not apply if especially specified.

The original strain and seed virus shall be stored frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

2.2 Materials for production

2.2.1 Embryonated chicken eggs

Embryonated chicken eggs aged 10–12 days shall be used.

2.3 Bulk material

2.3.1 Virus cultivation

Each strain of virus shall be separately inoculated into the allantoic cavities of embryonated chicken eggs, and the inoculated eggs shall be incubated. The allantoic fluid containing the propagated virus shall be harvested to serve as the virus suspension of each strain.

The virus suspension shall be tested as specified in 3.1.

2.3.2 Virus purification and concentration

Each virus suspension shall be purified and concentrated by the ultracentrifugal method, alcohol precipitation method, or other methods approved as suitable. In this case, a stabilizer approved as suitable may be added.

2.3.3 Inactivation

Each purified and concentrated virus suspension shall be inactivated by the addition of formalin to serve as the virus suspension of each strain.

The inactivated virus suspensions shall be tested as specified in 3.2.

2.3.4 Bulk material

Virus suspension shall be mixed and diluted with a dilution approved as suitable to adjust the concentration to serve as the bulk material.

The bulk material shall be tested as specified in 3.3.

2.4 Final bulk

The bulk material shall be mixed to serve as the final bulk. In this case, a stabilizer approved as suitable may be added.

2.5 Final product

The final bulk shall be dispensed into small containers to serve as the final products.

The final products shall be tested as specified in 3.4.

3 Test methods

3.1 Test on virus suspension

3.1.1 Hemagglutination titer test

3.1.1.1 Materials

3.1.1.1.1 Test materials

A series of two-fold dilutions of the test article shall be prepared using physiological saline. Each dilution shall be used as the test material.

3.1.1.2 Test procedures

To each 0.4 mL portion of the test material, add 0.4 mL portion of chicken red blood cells adjusted to 0.5 vol% and allow to react for 60 minutes.

3.1.1.3 Judgment

The highest dilution of test article that causes the hemagglutination is considered the hemagglutination titer.

The hemagglutination titer of the test article shall be within its specific range.

3.2 Test on inactivated virus suspension

3.2.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.2 Inactivation test

3.2.2.1 Materials

3.2.2.1.1 Injection materials

The test article shall be used as the injection material.

3.2.2.1.2 Embryonated chicken eggs

Eggs aged 9–10 days shall be used as specified in 1.1 in the Materials for Live Vaccine Production.

3.2.2.2 Test procedures

The injection material shall be inoculated at a dose of 0.2 mL into the allantoic cavities of at least six embryonated chicken eggs. The inoculated eggs shall be incubated at 32℃ to 36℃. The allantoic fluid shall be passaged every three days to the second generation. To the second-passaged allantoic fluid, red blood cells given in 3.1.1.2 shall be added and observed.

If the allantoic fluid of each egg shows hemagglutination, equal volumes of these allantoic fluids shall be mixed and passaged to the third generation. The third-passaged allantoic fluid shall be retested in a similar manner and then judged.

3.2.2.3 Judgment

If no hemagglutination is observed, the test article shall be judged to be negative for active virus.

No active virus shall be observed in the test article.

3.3 Tests on bulk material

3.3.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.3.2 CCA test

3.3.2.1 Materials

The test article and standard influenza vaccine (for CCA) (Note 1) shall be used as the materials.

3.3.2.2 Test procedures

The CCA value of the test article shall be determined by the modified Miller-Stanley's method.

3.3.2.3 Judgment

The CCA value in 1 mL of the test article shall be within its specific range.

3.4 Tests on final product

3.4.1 Properties test

When the test is performed as specified in the Properties Test of the General Tests, the final product shall be a liquid or a homogeneous suspension with a specific color, and no foreign matter and foreign odor shall be observed. The property of product per small container shall be uniform.

3.4.2 Test for pH

When the test is performed as specified in the Test for pH of the General Tests, the pH shall have an intrinsic value.

3.4.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.4.4 Test for formalin content

For products added with formalin~~,~~ when the test given in the Test for Formalin Content of the General Tests is applied, the formalin content shall be no higher than 0.1 vol%.

3.4.5 Test for thimerosal content

For products added with thimerosal, the test given in the Test for Thimerosal Content of the General Tests shall apply.

3.4.6 Test for protein nitrogen content

When the test is performed as specified in the Test for Protein Nitrogen Content of the General Tests, the protein nitrogen content shall be 50 μg or lower in 1 mL.

3.4.7 Test for freedom from abnormal toxicity

The test given in the Test for Freedom from Abnormal Toxicity of the General Tests shall apply.

3.4.8 Potency test

3.4.8.1 Materials

3.4.8.1.1 Injection materials

The test sample shall be diluted ten-fold with phosphate-buffered saline to serve as the injection material.

3.4.8.1.2 Test animals

Mice aged 4 weeks shall be used.

3.4.8.1.3 Hemagglutination antigens

Hemagglutination antigens of each virus strain contained in the test sample shall be used.

3.4.8.2 Test procedures

Twenty test animals inoculated with 0.5 mL portions of injection materials into the peritoneal cavities, and then divided into four groups. Sera obtained after 14 days shall be pooled for each group to perform a hemagglutination inhibition test. The sera to be tested shall be prepared by RDE, trypsin, potassium periodate, or a method approved as suitable and make two-fold dilutions. To 0.2 mL of each dilution, a 0.2 mL portion of hemagglutination antigens containing 8 units per 0.4 mL shall be added and treated at 37℃ for 60 minutes. To the treated mixtures, 0.4 mL portions of red blood cells given in 3.1.1.2 shall be added, allowed to stand for 60 minutes, and observed for the presence of hemagglutination.

3.4.8.3 Judgment

The highest dilution of the pooled sera that causes the inhibition of hemagglutination is considered the hemagglutination inhibition titer. In at least two of four groups, the hemagglutination inhibition titer shall be no less than 8.

4 Storage and expiry date

The expiry date shall be one year and a half after the manufacturing unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

Note 1 Standard influenza vaccine (for CCA)

A standard provided by the National Veterinary Assay Laboratory that contains 1,000 CCA of virus in 1 mL

**Japanese Encephalitis Vaccine, Inactivated, Seed**

1 Definition

Japanese encephalitis, vaccine, inactivated, seed is a vaccine of inactivated virus suspension obtained by propagating Japanese encephalitis virus that meets the Seed Lot Specifications in cell lines that meet the said specifications.

2 Production methods

2.1 Virus strain used for production

2.1.1 Name

Japanese encephalitis virus Nakayama-Yakken strain or strain approved as equivalent thereof

2.1.2 Properties

This virus strain shall be propagated in primary cells of swine kidneys, and goose, day-old chick and pigeon red blood cells shall be hemagglutinated.

2.1.3 Master seed virus

2.1.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in cells approved as suitable, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored frozen at -60ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.

The master seed virus shall not be passaged for purposes other than the production of vaccines.

The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.4 Working seed virus

2.1.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in cells approved as suitable.

The working seed virus shall be stored frozen at -60ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.5 Production seed virus

2.1.5.1 Propagation and storage

The production seed virus shall be propagated in cells approved as suitable.

The production seed virus shall be stored frozen at -60ºC or lower or stored freeze-dried at 5ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.2 Materials for production

2.2.1 Cell line

Vero cells or cell lines approved as suitable for production shall be used.

2.2.2 Culture medium

The culture medium approved as suitable for production shall be used.

2.2.3 Master cell seeds

2.2.3.1 Preparing, storing, and maximum passage number allowed for obtaining production cell seeds

The master cell seeds shall be propagated in a culture medium in 2.2.2, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master cell seeds shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower.

The master cell seeds shall be tested as specified in 3.2.1.

The master cell seeds shall not be passaged for purposes other than production or testing of vaccines.

The master cell seeds shall not be passaged more than 20 times to obtain the production cell seeds.

2.2.4 Working cell seeds

2.2.4.1 Propagation, passage, and storage

The working cell seeds shall be propagated and passaged using culture medium in 2.2.2.

The working cell seeds shall be stored frozen at -70ºC or lower.

The working cell seeds shall be tested as specified in 3.2.2.

2.2.5 Production cell seeds

2.2.5.1 Propagation and storage

The production cell seeds shall be propagated using culture medium in 2.2.2.

The production cell seeds shall be stored frozen at -70°C or lower when it needs to be stored.

The production cell seeds shall be tested as specified in 3.2.3 if they need to be stored.

2.3 Bulk material

2.3.1 Culture of production cell seeds

The cultured cells obtained in one session shall be regarded as individual cultured cells. No abnormalities shall be detected in the cultured cells prior to the inoculation of the virus.

2.3.2 Virus cultivation

The production seed virus shall be cultivated in the cells in 2.3.1. The filtrate, supernatant after centrifugation, or concentrate of the culture medium collected from individual cultured cells at the peak of virus propagation shall serve as the virus suspension.

The virus suspension shall be tested as specified in 3.3.

2.3.3 Inactivation

The virus suspension shall be inactivated by the addition of formalin or inactivator approved as suitable and shall serve as the inactivated virus suspension.

The inactivated virus suspension shall be tested as specified in 3.4.

2.3.4 Bulk material

The inactivated virus suspension shall be mixed to make the final bulk. The bulk material shall be tested as specified in 3.5.

2.4 Final bulk

The bulk material shall be mixed, diluted with a dilution approved as suitable to adjust its concentration and shall serve as the final bulk.

In this case, preservatives approved as suitable may be added.

2.5 Final product

The final bulk shall be dispensed into small containers to serve as the final product.

The final product shall be tested as specified in 3.6.

3 Test methods

3.1 Tests on virus strain used for production

3.1.1 Tests on master seed virus

3.1.1.1 Identification test

The tests given in 1.4.2.3.1.1 in the Seed Lot Specifications shall apply.

3.1.1.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.1.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.1.4 Test for freedom from extraneous viruses

3.1.1.4.1 Common test for freedom from extraneous viruses

The tests given in 1.1, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.2 Test for freedom from specific viruses

3.1.1.4.2.1 General test for freedom from specific viruses

For the Equine infectious anemia virus, Porcine reproductive and respiratory syndrome virus, and Endogenous retrovirus (Types C and D particles) in a test using cells derived from monkeys, the test given in 1.1 and 3.1.1 of the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.2.2 Individual test for freedom from specific viruses

For the Classical swine fever virus, Porcine circovirus, Bovine viral diarrhea virus, Rotavirus, and Rabies virus, the test given in 1.1, 3.2.3, 3.2.4, 3.2.5, 3.2.7 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.2 Tests on working seed virus

3.1.2.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.2.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.3 Tests on production seed virus

3.1.3.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.3.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2 Tests on cell line

3.2.1 Tests on master cell seeds

3.2.1.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.1.1 in the Seed Lot Specifications shall apply.

3.2.1.2 Test for identification of the animal species of the cell

The tests given in 2.1.4.2.1.2 in the Seed Lot Specifications shall apply.

3.2.1.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.1.4 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.1.5 Test for freedom from extraneous viruses

3.2.1.5.1 Common test for freedom from extraneous viruses

The tests given in 1.2, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.5.2 Test for freedom from specific viruses

3.2.1.5.2.1 General test for freedom from specific viruses

For the Equine infectious anemia virus, Porcine reproductive and respiratory syndrome virus, and Endogenous retrovirus (Types C and D particles), the test given in 1.2 and 3.1.1 of the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.5.2.2 Individual test for freedom from specific viruses

For the Classical swine fever virus, Porcine circovirus, Bovine viral diarrhea virus, Rotavirus, Japanese encephalitis virus, and Rabies virus, the test given in 1.2, 3.2.3, 3.2.4, 3.2.5, 3.2.7 and 3.2.9 of the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.6 Test for karyological (chromosomal) characterization

The tests given in 2.1.4.2.1.6.1 in the Seed Lot Specifications shall apply.

3.2.1.7 Test for freedom from tumorigenicity

The tests given in 2.1.4.2.1.7 in the Seed Lot Specifications shall apply.

3.2.2 Tests on working cell seeds

3.2.2.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.2.1 in the Seed Lot Specifications shall apply.

3.2.2.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.2.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.3 Tests on production cell seeds

3.2.3.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.3.1 in the Seed Lot Specifications shall apply.

3.2.3.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.3.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.3 Tests on virus suspension

3.3.1 Test for virus content

The test shall be performed through the inoculation test in mice or inoculation test in cultured cells.

3.3.1.1 Inoculation test in mice

3.3.1.1.1 Materials

3.3.1.1.1.1 Test materials

The test article shall be diluted ten-fold with phosphate-buffered saline or diluent solution approved as suitable, and each level dilution shall serve as the test material.

3.3.1.1.1.2 Test animals

Mice aged approximately 3 weeks shall be used.

3.3.1.1.2 Test procedures

A 0.03 mL each of the test materials shall be injected into the brain of at least four test animals and observed for 14 days.

3.3.1.1.3 Judgment

Mice that showed encephalitis symptoms and died shall be judged to be infected to calculate LD50.

The virus content in 1 mL shall be 107.5LD50 or higher.

3.3.1.2 Inoculation test in cultured cells

3.3.1.2.1 Materials

3.3.1.2.1.1 Test materials

The test article shall be diluted ten-fold with the virus growth medium (Note 1), and each level dilution shall serve as the test material.

3.3.1.2.1.2 Cultured cells

Primary cultured cells of chicken embryo in accordance with 2.1.1 of the Materials for Live Vaccine Production or cultured cells approved as suitable shall be cultured in small test portions, and cells that are grown as monolayers shall be used.

3.3.1.2.2 Test procedures

The 0.1 mL portions of the test material shall be inoculated into at least four portions of cultured cells and allowed to stand for adsorption at 37ºC for 60 minutes. The 0.5 mL portions of the virus growth medium shall be added, cultured at 37ºC for seven days, and observed.

3.3.1.2.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in 1 mL shall be 107.5TCID50 or higher.

3.4 Tests on inactivated virus suspension

3.4.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.4.2 Inactivation test

3.4.2.1 Materials

3.4.2.1.1 Injection materials

The test article shall be used as the injection materials.

3.4.2.1.2 Test animals

Mice aged 3 weeks shall be used.

3.4.2.2 Test procedures

A 0.03 mL each of the injection materials shall be injected into the brain of 10 test animals, and observed for 14 days.

3.4.2.3 Judgment

If there are no test animals that showed encephalitis symptoms and died, the test article shall be judged to be negative for active virus.

No active virus shall be observed in the test article.

3.5 Tests on bulk material

3.5.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.6 Tests on final product

3.6.1 Properties test

When the test is performed as specified in the Properties Test of the General Tests, the final product shall be a liquid with a specific color or a homogeneous suspension, and no foreign matter and foreign odor shall be observed. The property of the product per small container shall be uniform.

3.6.2 Test for pH

When the test is performed as specified in the Test for pH of the General Tests, the pH shall have an intrinsic value.

3.6.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.6.4 Test for thimerosal content

For products added with thimerosal, the test given in the Test for Thimerosal Content of the General Tests shall apply.

3.6.5 Test for formalin content

For products added with formalin when the test given in the Test for Formalin Content of the General Tests is applied, the formalin content shall be no higher than 0.2 vol%.

3.6.6 Test for freedom from abnormal toxicity

The test given in the Test for Freedom from Abnormal Toxicity of the General Tests shall apply.

3.6.7 Potency test

3.6.7.1 Materials

3.6.7.1.1 Injection materials

The test sample diluted ten-fold with phosphate-buffered saline shall be used as the injection materials.

3.6.7.1.2 Test animals

Mice aged 2–3 weeks shall be used.

3.6.7.2 Challenge virus

The Japanese encephalitis virus Nakayama-Yakken strain or strain approved as suitable (Note 2) shall be used.

3.6.7.3 Test procedures

Thirty and sixty animals shall be assigned to the test group and control group, respectively.

On Day 1 and Day 4 of the test, 0.1 mL each of the injection materials shall be injected intraperitoneally into the animals in the test group.

On Day 8 of the test, a 0.2 mL portion of challenge virus shall be injected intraperitoneally into 30 animals each of the test group and control group. Then, other 30 animals in the control group shall be divided into three groups (10 animals per group), and these groups shall be injected intraperitoneally with a 0.2 mL portion of the challenge virus diluted 10-fold, 100-fold, and 1,000-fold, respectively. The test group and control group shall be observed for 14 days.

3.6.7.4 Judgment

Animals that died showing encephalitis symptoms and animals that survived but still showing encephalitis symptoms shall be considered dead, and the mortality rate of each group and LD50 of challenge virus shall be calculated.

The tolerability of the test group must be more than 40%. In this case, the mortality rate of the control group injected with challenge virus must be more than 90%, and the challenge virus content must be less than 103LD50 in 0.2 mL.

4 Storage and expiry date

The expiry date shall be one year after the manufacturing unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

Note 1 Virus growth medium

1,000 mL consists of the following:

|  |  |
| --- | --- |
| Tryptose phosphate broth | 2.95 g |
| Bovine serum or goat serum | 0–50 mL |
| Eagle’s MEM | Residual quantity |

The pH shall be adjusted to 7.0–7.6 with sodium hydrogen carbonate.

Minimal amounts of antibiotics may be added.

Note 2 Challenge virus

The Japanese encephalitis virus Nakayama-Yakken strain or strain approved as suitable shall be inoculated into the brain of mice aged 3-4 weeks, sampled at the peak of disease onset, and diluted with phosphate-buffered saline or dilutions approved as suitable to serve as the 10-fold emulsion.

The supernatant obtained by centrifuging this solution shall be used as the challenge virus.

**Mycoplasma gallisepticum Vaccine, Live, Seed**

1 Definition

Mycoplasma gallisepticum vaccine, live, seed is a freeze-dried vaccine of culture bacteria medium of attenuated *Mycoplasma gallisepticum* that meets the Seed Lot Specifications.

2 Production methods

2.1 Bacterial strain used for production

2.1.1 Name

*Mycoplasma gallisepticum* G210 strain or strain approved as equivalent thereof

2.1.2 Properties

This bacterial strain shall agglutinate chicken red blood cells and have the immunogenicity to prevent *Mycoplasma gallisepticum.*

2.1.3 Master seed bacteria

2.1.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed bacteria shall be propagated in a medium approved as suitable and dispensed into storage containers.

The dispensed master seed bacteria shall be given a specific manufacturing number or manufacturing code and stored frozen at -70ºC or lower or stored freeze-dried at 5ºC or lower.

The master seed bacteria shall be tested as specified in 3.1.1.

The master seed bacteria shall not be passaged for purposes other than the production of vaccines. The master seed bacteria shall not be passaged more than 10 times to obtain the final product. However, the number of passages shall apply if approved by the Minister of Agriculture, Forestry and Fisheries.

2.1.4 Working seed bacteria

2.1.4.1 Propagation, passage, and storage

The working seed bacteria shall be propagated and passaged in medium approved as suitable for production.

The working seed bacteria shall be frozen at -70ºC or lower or be stored freeze-dried at 5ºC or lower.

The working seed bacteria shall be tested as specified in 3.1.2.

2.1.5 Production seed bacteria

2.1.5.1 Propagation and storage

The production seed bacteria shall be propagated in a medium approved as suitable for production.

The production seed bacteria shall be prepared before use for each vaccine production.

2.2 Materials for production

2.2.1 Medium

A medium approved as suitable for production shall be used.

2.3 Bulk material

2.3.1 Cultivation

The production seed bacteria inoculated into a medium and cultured shall serve as the cultured bacterial medium. This may be concentrated by methods approved as suitable.

The cultured bacterial medium shall be tested as specified in 3.2.

2.3.2 Bulk material

Bulk material shall be obtained by adjusting, if necessary, the concentration of bacteria in the cultured bacterial medium. A stabilizer approved as suitable may be added.

The bulk material shall be tested as specified in 3.3.

2.4 Final bulk

A stabilizer approved as appropriate shall be added to the bulk material to serve as the final bulk.

2.5 Final product

The final bulk shall be dispensed into small containers and freeze-dried to serve as the final product.

The final product shall be tested as specified in 3.4.

3 Test methods

3.1 Tests on bacterial strain used for production

3.1.1 Tests on master seed bacteria

3.1.1.1 Identification test

The tests given in 1.4.2.2.1.1 in the Seed Lot Specifications shall apply.

3.1.1.2 Test for freedom from contaminant microorganisms

The test given in the Sterility Test of the General Tests shall apply.

3.1.1.3 Target animal immunogenicity test

The test given in the Target Animal Immunogenicity Test of the General Tests shall apply.

3.1.1.4 Target animal safety test

The test given in the Target Animal Safety Test of the General Tests shall apply.

3.1.1.5 Test for absence of reversion virulence

The test given in the Test for Absence of Reversion to Virulence of the General Tests shall apply.

3.1.2 Tests on working seed bacteria

3.1.2.1 Test for freedom from contaminant microorganisms

The test given in the Sterility Test of the General Tests shall apply.

3.2 Test on cultured bacterial medium

3.2.1 Test for freedom from contaminant microorganisms

The test given in the Sterility Test of the General Tests shall apply.

3.3 Tests on bulk material

3.3.1 Test for freedom from contaminant microorganisms

The test given in the Sterility Test of the General Tests shall apply.

3.3.2 Viable count test

3.3.2.1 Materials

3.3.2.1.1 Test materials

The test article shall be diluted ten-fold with the dilution medium (Note 1) or medium approved as suitable, and each level dilution shall serve as the test material.

3.3.2.1.2 Medium

Dilution medium and agar medium (Note 2) or medium approved as suitable shall be used.

3.3.2.2 Test procedures

A 25 μL portion of the test material shall be inoculated into two agar plates or cultured by the pour plate culture method in an air containing 5 vol% carbon dioxide at 37ºC for 14 days.

3.3.2.3 Judgment

Calculate the viable count from the number of colonies formed. The viable count of the test material per 1 mL shall be 109.2 or greater. This number shall not apply if otherwise specified.

3.4 Tests on final product

3.4.1 Properties test

When the test is performed as specified in the Properties Test of the General Tests, the final product shall be dried substances with a specific color. The dissolved product shall be a liquid with a specific color, and no foreign matter and foreign odor shall be observed. The property of the product per small container shall be uniform.

3.4.2 Vacuum degree test

The test given in the Vacuum Degree Test of the General Tests shall apply.

3.4.3 Test for moisture content

The test given in the Test for Moisture Content of the General Tests shall apply.

3.4.4 Test for freedom from contaminant microorganisms

The test given in the Sterility Test of the General Tests shall apply.

3.4.5 Viable count test

When the test is performed as specified in 3.3.2, the viable count of the test sample per bird shall be 106.9 or greater. This number shall not apply if otherwise specified.

3.4.6 Identification test

This test may be omitted on products for which it is not specified.

3.4.6.1 Materials

3.4.6.1.1 Test materials

Dissolve the quantity of test samples for 1,000 birds in 30 mL of phosphate-buffered saline or diluent solution.

3.4.6.1.2 *Mycoplasma gallisepticum* positive serum

*Mycoplasma gallisepticum* positive serum (Note 3) shall be used.

3.4.6.2 Test procedures

Mix 30 μL of the test material and 30 μL of *Mycoplasma gallisepticum* positive serum on a reaction glass plate and observe the reaction while tilting the plate back and forth, left and right.

3.4.6.3 Judgment

Aggregated granules must be observed in one minute.

3.4.7 Marker test

This test may be omitted on products for which it is not specified.

3.4.7.1 Materials

3.4.7.1.1 Inoculation materials

The test materials given in 3.4.5 shall be used.

3.4.7.1.2 Embryonated chicken eggs

The embryonated chicken eggs aged six days in 1.1 in the Materials for Live Vaccine Production shall be used.

3.4.7.2 Test procedures

A 0.1 mL each of the test materials shall be injected into the yolk sac of 10 embryonated chicken eggs, cultured at 37ºC for 14 days, and observed.

3.4.7.3 Judgment

Chicken embryos in which the specific lesions are observed shall be regarded as infected, and EID50 shall be calculated. Of them, the embryos that died within 24 hours of inoculation shall be excluded from the calculation. The viable count per EID50 must be 105 or greater when calculated from the viable count obtained by the test given in 3.4.5.

3.4.8 Safety test

3.4.8.1 Materials

3.4.8.1.1 Inoculation materials

Dilute the test sample with phosphate-buffered saline or diluent so that a 0.1 mL portion of the dilution contains the quantity of test sample for 10 birds to serve as the inoculation materials.

3.4.8.1.2 Test animals

The chickens aged 2–4 weeks derived from 1.1 in the Materials for Live Vaccine Production.

3.4.8.2 Test procedures

Ten and five test animals shall be assigned to the test group and control group, respectively.

Inoculate 0.1 mL of inoculation materials ophthalmically into the test group and observe both groups for three weeks. On the final day of the test, the animals shall be autopsied and observed for the presence of lesions in the nasal cavity, suborbital sinus, trachea, and air sacs.

3.4.8.3 Judgment

During the period of observation, no clinical abnormalities shall be observed in the test and control groups. No abnormalities shall be observed in the autopsy.

3.4.9 Potency test

3.4.9.1 Materials

3.4.9.1.1 Inoculation material

Dilute the test sample with phosphate-buffered saline or diluent so that a 0.03 mL of the dilution contains the quantity of test sample for one bird to serve as the inoculation material.

3.4.9.1.2 Test animals

The chickens aged 2–4 weeks derived from 1.1 in the Materials for Live Vaccine Production shall be used.

3.4.9.1.3 Agglutination antigen

Inactivated antigen against *Mycoplasma gallisepticum* (Note 4) shall be used.

3.4.9.2 Test procedures

Ten and three animals shall be assigned to the test group and control group, respectively.

Inoculate 0.03 mL portions of inoculation material ophthalmically into the test group. After 4 weeks, the sera of individual animals obtained from the test and control groups are subjected to an agglutination reaction test using the agglutination antigen.

3.4.9.3 Judgment

At least 70% of the test group must be positive for agglutination antibodies in three minutes. In this case, all of the control group must be negative for agglutination antibodies.

4 Storage and expiry date

The expiry date shall be three years and three months after the manufacturing unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

Note 1 Dilution medium

1,000 mL consists of the following:

|  |  |
| --- | --- |
| Proteose peptone | 7.4 g |
| Yeast extract | 2.5 g |
| Dextrose | 4.0 g |
| Sodium chloride | 5.0 g |
| Sodium dihydrogen phosphate dihydrate | 1.8 g |
| Phenol red | 15 mg |
| Water | Residual quantity |

After adjusting the pH to 8.0, sterilize by filtration.

Note 2 Agar medium

The agar medium used is obtained by adding 1.0 g of bactoagar to 90.0 mL of dilution medium from which phenol red is removed, sterilizing by autoclaving at 121ºC for 15 minutes, cooling to approximately 50ºC, and adding 10 mL of swine serum.

Note 3 *Mycoplasma gallisepticum* positive serum

A serum obtained by immunizing the chickens in 1.1 in the Materials for Live Vaccine Production with the R strain of *Mycoplasma gallisepticum* or strain approved as suitable and showing positive serum plate agglutination reactions. It shall have the potency to show agglutination in a minute.

Note 4 Inactivated antigen of *Mycoplasma gallisepticum*

Inactivated antigen of *Mycoplasma gallisepticum* used is obtained by harvesting bacteria from the liquid culture medium of *Mycoplasma gallisepticum* S6 strain and diluting with phosphate-buffered saline so that the viable count is 1010 per mL. Then, they shall be inactivated with thimerosal and stained with crystal violet.

Agglutination is observed in three minutes in a serum plate agglutination reaction test using sera immunized with *Mycoplasma gallisepticum* S6 strain; however, no agglutination is observed in three minutes in the test using serum obtained from non-immunized control chickens or chickens immunized with *Mycoplasma* *synoviae*.

**Rabies (tissue culture) Vaccine, Inactivated, Seed**

1 Definition

Rabies (tissue culture) vaccine, inactivated, seed is a vaccine of purified and inactivated virus suspension obtained by propagating Rabies virus adapted to tissue culture that meets the Seed Lot Specifications in cell lines that meet the said specifications.

2 Production methods

2.1 Virus strain used for production

2.1.1 Name

Cell culture adapted RC･HL strain of Rabies virus

2.1.2 Properties

Injection of this strain into the brain of suckling mice aged 3 days or less causes the onset of the disease and death of the infected animals; however, the injection of the strain into the brain of mice aged 3 weeks or older, guinea pigs weighing approximately 300 g, rabbits weighing approximately 1.5 kg and dogs aged 1.5 months have almost no pathogenic effects.

The strain propagates in HmLu cells with CPE.

2.1.3 Master seed virus

2.1.3.1 Preparing, storing, and maximum passage number allowed for obtaining final products

The master seed virus shall be propagated in HmLu cells, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master seed virus shall be given a specific manufacturing number or manufacturing code and stored freeze-dried at 5ºC or lower.

The master seed virus shall be tested as specified in 3.1.1.

The master seed virus shall not be passaged for purposes other than the production of vaccines. The master seed virus shall not be passaged more than five times to obtain the final product.

2.1.4 Working seed virus

2.1.4.1 Propagation, passage, and storage

The working seed virus shall be propagated and passaged in HmLu cells.

The working seed virus shall be stored freeze-dried at 5ºC or lower.

The working seed virus shall be tested as specified in 3.1.2.

2.1.5 Production seed virus

2.1.5.1 Propagation, passage, and storage

The production seed virus shall be propagated in HmLu cells.

The production seed virus shall be stored frozen at -70ºC or lower when it needs to be stored.

The production seed virus shall be tested as specified in 3.1.3 if it needs to be stored.

2.2 Materials for production

2.2.1 Cultured cells

HmLu cells shall be used.

2.2.2 Culture medium

The culture medium approved as suitable for production shall be used.

2.2.3 Master cell seeds

2.2.3.1 Preparing, storing, and maximum passage number allowed for obtaining production cell seeds

The master cell seeds shall be propagated in a culture medium in 2.2.2, prepared in consecutive processes, and dispensed into storage containers.

The dispensed master cell seeds shall be given a specific manufacturing number or manufacturing code and stored frozen at -100ºC or lower.

The master cell seeds shall be tested as specified in 3.2.1.

The master cell seeds shall not be passaged for purposes other than production or testing of vaccines. The master cell seeds shall not be passaged more than 20 times to obtain the production cell seeds. When using the suspension culture method, approximately 3-fold increase in cell count than increase in the population doubling time shall be regarded as passage of one generation.

2.2.4 Working cell seeds

2.2.4.1 Propagation, passage, and storage

The working cell seeds shall be propagated and passaged using culture medium in 2.2.2.

The working cell seeds shall be stored frozen at -100ºC or lower.

The working cell seeds shall be tested as specified in 3.2.2.

2.2.5 Production cell seeds

2.2.5.1 Propagation, passage, and storage

The production cell seeds shall be propagated using culture medium in 2.2.2.

The production cell seeds shall be stored frozen at -100°C or lower when it needs to be stored.

The production cell seeds shall be tested as specified in 3.2.3 if they need to be stored.

2.3 Bulk material

2.3.1 Culture of production cell seeds

In the monolayer culture method, cells obtained and cultured in one session shall be regarded as individually cultured cells, and in the suspension culture method, cells cultured in the final fermenter shall be regarded as individually cultured cells, respectively. No abnormalities shall be detected in the cultured cells prior to inoculation of the virus.

2.3.2 Virus cultivation

The production seed virus shall be inoculated into the cultured cells, cultured at 32ºC to 34ºC, and harvested at the peak of CPE or G protein production. Centrifugal supernatant or filtrate of the culture medium obtained shall serve as the virus suspension.

The virus suspension shall be tested as specified in 3.3.

2.3.3 Purification

The virus suspension shall be purified and concentrated with macrogol or in a manner approved as suitable to serve as the purified virus suspension. In the suspension culture method, purification may be followed by the inactivation process given in 2.3.4.

2.3.4 Inactivation

The purified virus suspension shall be inactivated with β-propiolactone or in a manner approved as suitable to serve as the inactivated virus suspension.

The inactivated virus suspension shall be tested as specified in 3.4.

2.3.5 Adjustment of bulk material

Inactivated virus suspension shall be diluted with phosphate-buffered saline to adjust its concentration and serve as the bulk material. To inactivated virus suspension or inactivated and purified virus suspension, in the suspension culture method, add preservatives approved as suitable to serve as the bulk material.

The bulk material shall be tested as specified in 3.5.

2.4 Final bulk

The bulk material shall be mixed, diluted with phosphate-buffered saline to adjust its concentration in the suspension culture method, and serve as the final bulk. In this case, preservatives approved as suitable may be added.

2.5 Final product

The final bulk shall be dispensed into small containers to serve as the final product.

The final product shall be tested as specified in 3.6.

3 Test methods

3.1 Tests on virus strain used for production

3.1.1 Tests on master seed virus

3.1.1.1 Identification test

The tests given in 1.4.2.1.1.1 in the Seed Lot Specifications shall apply.

3.1.1.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.1.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.1.4 Test for freedom from extraneous viruses

3.1.1.4.1 Common test for freedom from extraneous viruses

The tests given in 1.1, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.2 Test for freedom from specific viruses

3.1.1.4.2.1 General test for freedom from specific viruses

For the Feline leukemia virus/Feline sarcoma virus and Lymphocytic choriomeningitis virus, the test given in 1.1 and 3.1.1 of the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.1.4.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Canine parvovirus, Feline panleukopenia virus, and Japanese encephalitis virus, the test given in 1.1, 3.2.5, 3.2.6 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.1.2 Tests on working seed virus

3.1.2.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.2.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.1.2.3 Efficiency and immunogenicity test

3.1.2.3.1 Tests using guinea pigs

3.1.2.3.1.1 Materials

3.1.2.3.1.1.1 Test materials

Dissolve the new and old working seed viruses in phosphate-buffered saline and adjust the concentrations so that the virus content in 1 mL is approximately 107.0TCID50. To the virus solutions, add β-Propiolactone so that the concentration is 0.0125 vol%, sensitize at 4ºC for 48 hours and inactivate the virus to serve as the test material.

3.1.2.3.1.1.2 Test animals

Guinea pigs weighing approximately 400 g shall be used.

3.1.2.3.1.1.3 Challenge virus

Rabies virus CVS strain (Note 1) shall be used. Adjust the concentration with physiological saline containing 2 vol% horse serum to be 10 LD50 of the virus in 0.2 mL when injecting into the masseter muscle of guinea pigs and serve as the challenge virus.

3.1.2.3.1.2 Test procedures

Inject the old and new test materials at a dose of 0.5 mL subcutaneously into the inner thigh of 10 test animals, respectively. These treated animals shall be regarded as the test groups. Another 10 animals shall be regarded as the untreated control group. On the 21st day after injection, inject 0.2 mL of challenge virus into masseter muscle of the three groups each, and observe the animals clinically for 14 days.

3.1.2.3.1.3 Judgment

The animals that did not develop the disease shall be regarded as tolerant, and the tolerability shall be calculated.

The tolerability of both groups shall be 70% or higher. In this case, the tolerability of the control group shall be 20% or lower.

3.1.2.3.2 Tests using dogs

3.1.2.3.2.1 Materials

3.1.2.3.2.1.1 Test materials

The test materials specified in 3.1.2.3.1.1.1 shall be used.

3.1.2.3.2.1.2 Test animals

Dogs aged approximately 4 months and negative for antibody against rabies virus shall be used.

3.1.2.3.2.2 Test procedures

Inject 1 mL portions of the old and new test material subcutaneously into three test animals each, and one month later, collect the blood from each animal, and measure the neutralizing antibody titer using the cell culture adapted RC-HL strain of rabies virus.

3.1.2.3.2.3 Judgment

The neutralizing antibody titers in both study groups must be greater than ten-fold geometric mean, respectively.

3.1.3 Tests on production seed virus

3.1.3.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.1.3.2 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2 Tests on cell line

3.2.1 Tests on master cell seeds

3.2.1.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.1.1 in the Seed Lot Specifications shall apply.

3.2.1.2 Test for identification of the animal species of the cell

The tests given in 2.1.4.2.1.2 in the Seed Lot Specifications shall apply.

3.2.1.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.1.4 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.1.5 Test for freedom from extraneous viruses

3.2.1.5.1 Common test for freedom from extraneous viruses

The tests given in 1.2, 2.1 and 2.2 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.5.2 Test for freedom from specific viruses

3.2.1.5.2.1 General test for freedom from specific viruses

For the Feline leukemia virus/Feline sarcoma virus and Lymphocytic choriomeningitis virus, the test given in 1.2 and 3.1.1 of the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.5.2.2 Individual test for freedom from specific viruses

For the Bovine viral diarrhea virus, Canine parvovirus, Feline panleukopenia virus, Japanese encephalitis virus, and Rabies virus, the test given in 1.2, 3.2.5, 3.2.6 and 3.2.9 in the Test for Freedom from Extraneous Viruses of the General Tests shall apply.

3.2.1.6 Test for karyological (chromosomal) characterization

The tests given in 2.1.4.2.1.6 in the Seed Lot Specifications shall apply.

3.2.1.7 Test for freedom from tumorigenicity

The tests given in 2.1.4.2.1.7 in the Seed Lot Specifications shall apply.

3.2.2 Tests on working cell seeds

3.2.2.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.2.1 in the Seed Lot Specifications shall apply.

3.2.2.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.2.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.2.3 Tests on production cell seeds

3.2.3.1 Test for confirmation of cell properties

The tests given in 2.1.4.2.3.1 in the Seed Lot Specifications shall apply.

3.2.3.2 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.2.3.3 Test for freedom from Mycoplasma contamination

The test given in the Test for Freedom from Mycoplasma Contamination of the General Tests shall apply.

3.3 Tests on virus suspension

3.3.1 Test for virus content

3.3.1.1 Materials

3.3.1.1.1 Test materials

The test article shall be diluted ten-fold with cell growth medium (Note 2) and each level dilution shall serve as the test material.

3.3.1.1.2 Cultured cells

Primary cultured cells of chicken embryo shall be used.

3.3.1.2 Test procedures

The 0.1 mL portions of the test material shall be inoculated into at least four portions (wells) of cultured cells and allowed to stand to culture at 37ºC for two days. On the second day, the culture medium shall be replaced by virus growth medium 1 (Note 3), allowed to stand to culture at 32ºC for eight days, and observed.

Note that if a 96-well microplate is used, each 25 μL portion of the test material shall be inoculated into 10 wells of cultured cells, then 0.2 mL portions of the virus growth medium 2 (Note 4) shall be added, cultured at 37ºC for 10 days and observed.

3.3.1.3 Judgment

The cultured cells in which CPE is observed shall be regarded as infected to calculate TCID50.

The virus content in the test article shall be 107.5 TCID50 or greater in 1 mL in the monolayer culture method, and 107.0 TCID50 or greater in 1 mL in the suspension culture method.

3.4 Tests on inactivated virus suspension

3.4.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.4.2 Inactivation test

3.4.2.1 Materials

3.4.2.1.1 Injection materials and inoculation materials

The test article shall be used as the injection material and inoculation material.

3.4.2.1.2 Test animals and cultured cells

Suckling mice aged three days or less and primary cultured cells of chicken embryo shall be used.

Note that if the primary cultured cells of chicken embryo are used, the 10 mL portions of the cells shall be dispensed into a culture bottle, and cells that are grown as monolayers of approximately 36 cm2 shall be used.

3.4.2.2 Test procedures

The 0.02 mL portions of the injection materials shall be injected into the brain of 10 test animals, and observed for 14 days.

The 2 mL portions of the inoculation material shall be inoculated into at least four portions of cultured cells and adsorbed at 37ºC for 60 minutes. The inoculation material shall be removed, the 10 mL portions of the virus growth medium 1 shall be added, allowed to stand to culture at 37ºC for 10 days, and observed.

3.4.2.3 Judgment

If the test animals show no symptoms of rabies virus, and the cultured cells in which no CPE is observed, the material shall be judged to be negative for active virus.

No active virus shall be observed in the test article.

3.5 Tests on bulk material

3.5.1 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.5.2 Potency test

3.5.2.1 Materials

The test article, reference vaccine (Note 5), antibody adsorption plate (Note 6), and enzyme labeled antibody (Note 7) shall be used.

3.5.2.2 Test procedures

To 6 mL of the test article, add 327.6 mg of disodium hydrogen phosphate dodecahydrate and 38.6 mg of potassium dihydrogen phosphate, and mix thoroughly by shaking. Dissolve the reference vaccine in the method specified by the National Veterinary Assay Laboratory. These test articles and reference vaccine shall be sonicated for 30–60 seconds each, and then centrifuged at approximately 200 G for 5 minutes. Previously filter each centrifuged supernatant through a membrane filter with a pore size of 450 nm pre-treated with phosphate-buffered saline containing 1 w/v% bovine serum albumin, and 4 mL of the filtrate obtained shall be gel-filtered (Note 8). Collect 8 mL of the filtrate containing the peak of the first fraction and prepare original (high dose), two-fold dilution (medium dose) and four-fold dilution (low dose) using the antigen diluting solution (Note 9). To two wells in an antibody adsorption plate, add 100 μL portions of antigen solutions to serve as the negative control wells. Seal the plate and allow to react at 37ºC for 60 minutes. After washing five times with washing solution (Note 10), add 100 μL portions of enzyme-labeled antibody to the wells each. The plate shall be sealed, and shielded from light, and allowed to react at 37ºC for 90 minutes. After washing five times with washing solution, add 100 μL of substrate chromogenic solution (Note 11) to the wells each. The plate shall be allowed to react at room temperature for 30 minutes avoiding light. Immediately after the reaction is completed, add the 50 μL portions of the stop solution (Note 12) to the wells each, and measure the absorbance at the main wavelength of 492 nm and at the reference-wavelength of 630 nm, respectively. The value obtained by subtracting the absorbance value of each negative control well from that of each well to which the test article and reference vaccine are added shall be defined as the absorbance value of each antigen. Measure the absorbance twice for each antigen solution after gel filtration and then calculate the relative titer of the test article against the reference vaccine from the absorbance values of the test article and the reference vaccine according to the relative titer calculation method (Note 13).

3.5.2.3 Judgment

The relative titer of the test article against the reference vaccine shall be 0.683 or higher.

3.6 Tests on final product

3.6.1 Properties test

When the test is performed as specified in the Properties Test of the General Tests, the final product shall be a liquid with a specific color, and no foreign matter and foreign odor shall be observed. The property of the product per small container shall be uniform.

3.6.2 Test for pH

When the test is performed as specified in the Test for pH of the General Tests, the pH shall have an intrinsic value.

3.6.3 Sterility test

The test given in the Sterility Test of the General Tests shall apply.

3.6.4 Test for thimerosal content

The test given in the Test for Thimerosal Content of the General Tests shall apply.

3.6.5 Test for macrogol content

When the test is performed as specified in the Test for Macrogol Content of the General Tests, the macrogol content shall be 0.5 mg or lower in 1 mL.

3.6.6 Test for protein nitrogen content

When the test is performed as specified in the Test for Protein Nitrogen Content of the General Tests, the protein nitrogen content shall be 100 μg or lower in 1 mL.

3.6.7 Safety test

3.6.7.1 Materials

3.6.7.1.1 Injection materials

The test samples shall be used as injection materials.

3.6.7.1.2 Test animals

Dogs weighing approximately 5–10 kg and cats weighing approximately 1 kg shall be used.

3.6.7.2 Test procedures

A 5 mL each of the injection materials shall be injected subcutaneously into two dogs and 2 mL each into two cats and observed clinically for 10 days.

3.6.7.3 Judgment

During the period of observation, no abnormalities shall be observed in the test group.

3.6.8 Potency test

The test given in 3.5.2 shall apply.

4 Storage and expiry date

The expiry date shall be two years after the manufacturing unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

Note 1 Rabies virus CVS strain

A sample of brain inoculated with rabies virus CVS strain in 3-week-old mice exhibiting symptoms. This sample is emulsified with physiological saline containing 2 vol% horse serum to make homogenates.

When this homogenate is administered into the masseter muscle of guinea pigs weighing approximately 400 g, the virus content shall be 102.5LD50 or higher in 0.2 mL.

Note 2 Cell growth medium

1,000 mL consists of the following:

|  |  |
| --- | --- |
| Tryptose phosphate broth | 2.95 g |
| Fetal bovine serum or calf bovine serum | 50 mL |
| Eagle’s MEM | Residual quantity |

The pH shall be adjusted to 7.1–7.3.

Minimal amounts of antibiotics may be added.

Note 3 Virus growth medium 1

1,000 mL consists of the following:

|  |  |
| --- | --- |
| Tryptose phosphate broth | 2.95 g |
| Eagle’s MEM | Residual quantity |

The pH shall be adjusted to 7.4–7.6.

Minimal amounts of antibiotics may be added.

Note 4 Virus growth medium 2

1,000 mL consists of the following:

|  |  |
| --- | --- |
| Glucose | 5 g |
| L-glutamine | 0.4 g |
| Fetal bovine serum or Calf serum | 5–25 mL |
| Eagle’s MEM | Residual quantity |

The pH shall be adjusted to 7.1–7.3.

Minimal amounts of antibiotics may be added.

Note 5 Reference vaccine

The reference vaccine used is obtained by adjusting the concentration of rabies (tissue culture) vaccine, inactivated, for reference provided by the National Veterinary Assay Laboratory as specified by the National Veterinary Assay Laboratory.

Note 6 Antibody adsorption plate

The antibody adsorption plate used is obtained by adding 100 μL portions of the dilution of purified monoclonal antibody against Rabies virus G protein of the concentration specified by the National Veterinary Assay Laboratory to each wells of a 96-well plate, sealing the plate, and allowing to adsorb at 37ºC for 18 hours.

The antibody adsorption plate shall be used after washing four times with phosphate-buffered saline.

Note 7 Enzyme labeled antibody

The enzyme labeled antibody used is obtained by adjusting the concentration of peroxidase-labeled monoclonal antibody against Rabies virus G protein with the following dilution solution to the concentration specified by the National Veterinary Assay Laboratory.

In 100 mL:

|  |  |
| --- | --- |
| Bovine serum albumin | 0.3 g |
| Polysorbate 20 | 0.05 mL |
| Phosphate-buffered saline | Residual quantity |

Sterilize by filtration through a membrane filter with a pore size of 450 nm.

Note 8 Gel filtration

Using a column specified by the National Veterinary Assay Laboratory, separate the fractions by liquid chromatography at a flow rate of 1 mL per minute with antigen diluting solution as the mobile phase.

The detection wavelength shall be 280 nm.

Note 9 Antigen diluting solution

In 1,000 mL:

|  |  |  |
| --- | --- | --- |
| Disodium hydrogen phosphate dodecahydrate | | 54.66 g |
| Potassium dihydrogen phosphate | 6.44 g | |
| Water | Residual quantity | |

After adjusting the pH to 7.2, filter through a membrane filter with a pore size of 450 nm.

Note 10 Washing solution

The washing solution used is obtained by adding polysorbate 20 to phosphate-buffered saline so that the concentration is 0.05 vol%.

Note 11 Substrate chromogenic solution

In 1,000 mL:

|  |  |
| --- | --- |
| Citric acid | 21.0 g |
| Anhydrous monobasic sodium phosphate | 28.4 g |
| Water | Residual quantity |

The dissolved solution shall be filtered through a membrane filter with a pore size of 450 nm. To 20 mL of the solution, add 10 mg of o-phenylenediamine dihydrochloride to dissolve. Immediately before use, add 5 μL of hydrogen peroxide (30).

Note 12 Stop solution

The stop solution used is obtained by adding 150 mL of sulfuric acid to 1,000 mL of water.

Note 13 Relative tier calculation method

1 Validity test

Multiply each absorbance value obtained by 1,000 and convert it to the ordinary logarithm. Evaluate the total values of the doses for the reference vaccine and the test article and calculate the following formula. The low, medium, and high doses of the reference vaccine shall be represented by S1, S2, and S3, respectively, and those of the test article shall be represented by T1, T2, and T3, respectively.

Difference between the test article and reference vaccine SPa ＝(S1+S2+S3)-(T1+T2+T3)

Linearity SPb ＝(S3-S1)+(T3-T1)

Curvilinearity SPc ＝[(S1+S3)-2S2]+[(T1+T3)-2T2]

Line non-parallelism SPb´＝(S3 -S1)-(T3-T1)

Curve non-parallelism SPc´＝[(S1+S3)-2S2]-[(T1+T3)-2T2]

If SPb obtained by the above formula is greater than or equal to 0.50, the absolute value of SPc is less than or equal to 0.86, that of SPb´ is less than or equal to 0.49, and that of SPc´ is less than or equal to 0.86: the validity test is met.

2 Calculation of relative tier

If the validity test given in 1 is judged as compliant, calculate the relative titer by the following formula:

M = - (4 × SPa × log 2) / (3 × SPb)

Furthermore, calculate the relative titer of the test article to the reference vaccine by finding the true number of M (inverse logarithm of M, 10M).

P= 10M