**Specifications**

**Materials for Live Vaccine Production**

Embryonated eggs, cultured cells, and bovine serum used as materials for live vaccine production shall comply with the following specifications. However, embryonated eggs and cultured cells used for seed lot products shall comply with the Seed Lot Specifications.

1 Embryonated eggs

1.1 Embryonated chicken eggs

Embryonated chicken eggs used for live vaccine production must be derived from SPF chicken flocks that have been confirmed to be free from the pathogens listed in Table 1 by the tests and treatment listed in the same table, or by the inspections and treatment approved as equivalent thereof.

1.2 Embryonated quail eggs

Embryonated quail eggs used for live vaccine production must be derived from SPF quail flocks that have been confirmed to be free from the pathogens listed in Table 2 by the tests and treatment listed in the same table, or by the inspections and treatment approved as equivalent thereof.

1.3 Embryonated duck eggs

Embryonated duck eggs used for live vaccine production must be derived from SPF duck flocks that have been confirmed to be free from the pathogens listed in Table 3 by the tests and treatment listed in the same table, or by the inspections and treatment approved as equivalent thereof.

2 Cultured cells

2.1 Cells derived from chicken embryos

2.1.1 Primary cultured cells of chicken embryos

Primary cultured cells of chicken embryos (chicken embryo fibroblasts) used for live vaccine production must be prepared from a chicken embryo derived from embryonated chicken eggs compliant with the specification given in 1.1.

2.1.2 Primary cultured cells of chicken embryo kidneys

Primary cultured cells of chicken embryo kidneys used for live vaccine production must be prepared from a chicken embryo kidney derived from embryonated chicken eggs compliant with the specification given in 1.1.

2.1.3 Primary cultured cells of chicken embryo livers

Primary cultured cells of chicken embryo livers used for live vaccine production must be prepared from a chicken embryo liver derived from embryonated chicken eggs compliant with the specification given in 1.1.

2.2 Cells derived from chickens

2.2.1 Primary cultured cells of chicken kidneys

Primary cultured cells of chicken kidneys used for live vaccine production must be prepared from a chicken kidney derived from embryonated chicken eggs compliant with the specification given in 1.1.

2.3 Cells derived from quail embryos

2.3.1 Primary cultured cells of quail embryos

Primary cultured cells of quail embryos (quail embryo fibroblasts) used for live vaccine production must be prepared from a quail embryo derived from embryonated quail eggs compliant with the specification given in 1.2.

2.4 Cells derived from duck embryos

2.4.1 Primary cultured cells of duck embryos

Primary cultured cells of duck embryos (duck embryo fibroblasts) used for live vaccine production must be prepared from a duck embryo derived from embryonated duck eggs compliant with the specification given in 1.3.

2.5 Cells derived from ducks

2.5.1 Primary cultured cells of duck kidneys

Primary cultured cells of duck kidneys used for live vaccine production must be prepared from a duck kidney derived from embryonated duck eggs compliant with the specification given in 1.3.

2.6 Cells derived from swine

2.6.1 Primary cultured cells of swine kidneys

Primary cultured cells of swine kidneys used for live vaccine production must be prepared from a lesion-free kidney removed from swine kept under health care for more than seven days prior to slaughter and have no abnormal findings including fever.

2.6.2 Primary cultured cells of swine testis

Primary cultured cells of swine testis used for live vaccine production must be prepared from a lesion-free testis removed from swine kept under health care for more than seven days prior to removal and have no abnormal findings including fever.

2.7 Cells derived from bovine

2.7.1 Primary cultured cells of bovine kidneys

Primary cultured cells of bovine kidneys used for live vaccine production must be prepared from a lesion-free kidney removed from bovine kept under health care for more than seven days prior to slaughter and have no abnormal findings including fever.

2.7.2 Primary cultured cells of bovine testis

Primary cultured cells of bovine testis used for live vaccine production must be prepared from a lesion-free testis removed from bovine kept under health care for more than seven days prior to slaughter and have no abnormal findings including fever.

3 Bovine serum

Bovine serum used for live vaccine production shall be separated from fresh blood of healthy bovine or bovine fetus, sterilized by filtration, dispensed, and heat-inactivated. When this is used as the test material and tested as specified in the Sterility Test and Test for Freedom from Mycoplasma Contamination of the General Tests and in 2.4.1 and 2.4.2 in the Test for Freedom from Extraneous Viruses for Live Vaccines and Sera of the General Tests, this test material shall comply with these tests. Bovine serum used for materials for production of live vaccines for cattle also shall comply with 2.8.1 in the Test for Freedom from Extraneous Viruses for Live Vaccines and Sera of the General Tests when tested as specified.

Table 1 Inspection and Treatment of SPF Chicken Flocks

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Pathogen | Antigen under inspection1） | Inspection timing and number of chickens inspected | | | | Inspection method2） | Treatment |
| 1st | | 2nd and subsequent | |
| Timing | Number of chickens (%) | Timing | Number of chickens (%) |
| Newcastle disease virus | Ishii strain | 8–12 weeks old | 20 | Every 3 months | 10 | HI | All the positive group and cohabitant group3） culling |
| Avian infectious bronchitis virus | M-41 strain | 〃 | 〃 | 〃 | 〃 | ELISA | 〃 |
| Avian leukosis virus | Sub-A, B | 〃 | 〃 | 〃 | 〃 | SN | 〃 |
| Avian encephalomyelitis virus | Van Roekel strain | 〃 | 〃 | 〃 | 〃 | ELISA | 〃 |
| Avian nephritis virus | G-4260 strain | 〃 | 〃 | 〃 | 〃 | FA | 〃 |
| Infectious laryngotracheitis virus | NS-175 strain | 〃 | 〃 | 〃 | 〃 | ELISA | 〃 |
| Reticuloendotheliosis virus | T strain | 〃 | 〃 | 〃 | 〃 | FA | 〃 |
| Marek's disease virus | JM strain | 〃 | 〃 | 〃 | 〃 | FA | 〃 |
| Infectious bursal disease virus | J1 strain | 〃 | 〃 | 〃 | 〃 | ELISA | 〃 |
| Avian reovirus | Uchida strain | 〃 | 〃 | 〃 | 〃 | DID | 〃 |
| Avian adenovirus | Ote strain | 〃 | 〃 | 〃 | 〃 | DID | 〃 |
| EDS-76 virus | JPA-1 strain | 〃 | 〃 | 〃 | 〃 | HI | 〃 |
| Avian influenza virus | 5331 strain | 〃 | 〃 | 〃 | 〃 | DID | 〃 |
| Chicken anemia virus | Gifu-1 strain | 〃 | 〃 | 〃 | 〃 | FA | 〃 |
| Turkey rhinotracheitis virus | MM-1 strain | 〃 | 〃 | 〃 | 〃 | FA | 〃 |
| Avian paramyxovirus | Yucaipa strain | 〃 | 〃 | 〃 | 〃 | HI | 〃 |
| *Haemophilus paragallinarum* type A | 221 strain | 〃 | 〃 | 〃 | 〃 | HI | 〃 |
| *Haemophilus paragallinarum* type C | S1 strain | 〃 | 〃 | 〃 | 〃 | HI | 〃 |
| *Salmonella pullorum* | 9-25 strain | 〃 | 〃 | 〃 | 〃 | AGG | 〃 |
| *Mycoplasma gallisepticum* | S6 strain | 〃 | 〃 | 〃 | 〃 | AGG | 〃 |
| *Mycoplasma synoviae* | WVU-1853 strain | 〃 | 〃 | 〃 | 〃 | AGG | 〃 |
| *Salmonella* (except for *Salmonella pullorum*) |  | 〃 | 〃 | 〃 | 〃 | Bacterial isolation | 〃 |
| Chickenpox virus |  | Everyday | 100 | Everyday | 100 | Clinical observation | Positive chickens culling |
|  |  |  |  |  |  |  |  |

Note All health conditions and abnormalities of chickens shall be completely recorded. For dead chickens, histopathological examinations and other associated examinations shall be performed.

1) The antigen may be substituted with another appropriate strain.

2) An equivalent inspection method may be applied, if available. The alternate inspection method to be applied shall be verified and guaranteed to ensure their validity. HI: Hemagglutination test ELISA: Enzyme-linked immunosorbent assay SN: Serum neutralization test FA: Fluorescence antibody test DID: Double immunodiffusion test AGG: Agglutination test

3) Cohabitant group refers to a group of animals not completely isolated from the positive group.

**Seed Lot Specifications**

1 Vaccine seeds

1.1 Range of passage numbers

To obtain the final product, the master seed virus shall not be passaged more than 5 times, the master seed bacteria shall not be passaged more than 10 times, the master seed coccidia shall not be passaged more than 10 times, unless otherwise approved by the Minister of Agriculture, Forestry and Fisheries.

1.2 Preparation method

Master seeds shall be prepared in accordance with the approved method in consecutive process to ensure their homogeneity and stability and prevent contamination.

Master seed virus shall be dispensed at a virus concentration at which it can be adequately neutralized by antiserum in the Test for Freedom from Extraneous Viruses.

1.3 Storage

Master seeds shall be stored under the approved conditions.

1.4 Origin of seeds and specifications and test methods

1.4.1 Records on origin

1.4.1.1 Origin

The following information on the origin of seeds shall be recorded: Method of isolation, place of isolation, timing of isolation, animal species of origin, and characteristics of isolates of viruses, bacteria, etc. isolated from animal species of origin.

If the master seed was distributed (or purchased), from whom it was distributed (or from whom it was purchased) and the time of distribution (or purchase) shall also be recorded.

If the seed was produced by the genetic recombination technology, the characteristics of the genetically modified microorganism (description of the host or the taxonomic species to which the host belongs, description of the donor nucleic acid, description of the vector, method of conditioning genetically modified microorganisms, method of identifying the genetically modified microorganism, differences from the host or the taxonomic species to which the host belongs, etc.) shall be recorded.

1.4.1.2 Passage history

With regard to passages after the isolation, the animals used, cultured cells, and media, cloning and attenuation methods shall be recorded. If the master seed virus has been distributed, its passage history before and after the distribution shall also be recorded.

1.4.2 Specifications and test methods

1.4.2.1 Live virus vaccines

1.4.2.1.1 Master seed virus

1.4.2.1.1.1 Identification test

The test shall be performed as specified in 1.4.2.1.1.1.1 or 1.4.2.1.1.1.2.

1.4.2.1.1.1.1 Fluorescent antibody assay

When the cultured cells inoculated with and without the test article are stained with a fluorescence-labeled antibody, respectively, fluorescence characteristic of the virus shall be detected in the cells inoculated with the test article but shall not be detected in those inoculated without it.

1.4.2.1.1.1.2 Serum neutralization test

During the growth of the test article using appropriate cultured cells, virus-specific cytopathogenic changes shall be detected, and the growth shall be neutralized by the specific antiserum.

1.4.2.1.1.2 Sterility test

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

1.4.2.1.1.3 Test for freedom from Mycoplasma contamination

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

1.4.2.1.1.4 Test for freedom from extraneous viruses

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

1.4.2.1.1.5 Target animal immunogenicity test

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

1.4.2.1.1.6 Target animal safety test

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

1.4.2.1.1.7 Test for absence of reversion to virulence

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

1.4.2.1.1.8 Test for stability confirmation of recombinant gene

If gene recombination technology is used for preparation of the master seed virus, the test given in the Test for Stability Confirmation of Recombinant Gene of the General Tests shall apply.

1.4.2.1.2 Working seed virus

1.4.2.1.2.1 Sterility test

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

1.4.2.1.2.2 Test for freedom from Mycoplasma contamination

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

1.4.2.1.3 Production seed virus

For storage, the following tests shall be performed:

1.4.2.1.3.1 Sterility test

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

1.4.2.1.3.2 Test for freedom from Mycoplasma contamination

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

1.4.2.2 Live bacteria vaccine

1.4.2.2.1 Master seed bacteria

1.4.2.2.1.1 Identification test

The test for identification of bacteria species shall be performed in compliance with an appropriate morphological characterization test method, biochemical characterization test method or other approved test methods.

1.4.2.2.1.2 Test for freedom from contaminant microorganisms

The test given in the respective sections of the parts of vaccine (seed lot product) shall apply. If no test method is given in the respective sections of the parts of vaccine (seed lot product), the test shall be performed in the approved manner.

1.4.2.2.1.3 Target animal immunogenicity test

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

1.4.2.2.1.4 Target animal safety test

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

1.4.2.2.1.5 Test for absence of reversion to virulence

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

1.4.2.2.1.6 Test for stability confirmation of recombinant gene

If gene recombination technology is used for preparation of the master seed bacteria, the test given in the Test for Stability Confirmation of Recombinant Gene of the General Tests shall apply.

1.4.2.2.2 Working seed bacteria

1.4.2.2.2.1 Test for freedom from contaminant microorganisms

The test given in the respective sections of the parts of vaccine (seed lot product) shall apply. If no test method is given in the respective sections of the parts of vaccine (seed lot product), the test shall be performed in the approved manner.

1.4.2.2.3 Production seed bacteria

For storage, the following tests shall be performed:

1.4.2.2.3.1 Test for freedom from contaminant microorganisms

The test given in the respective sections of the parts of vaccine (seed lot product) shall apply.

If no test method is given in the respective sections of the parts of vaccine (seed lot product), the test shall be performed in the approved manner.

1.4.2.3 Inactivated virus vaccine

1.4.2.3.1 Master seed virus

1.4.2.3.1.1 Identification test

The test shall be performed as specified in 1.4.2.3.1.1.1 or 1.4.2.3.1.1.2.

1.4.2.3.1.1.1 Fluorescent antibody assay

Stain the cultured cells inoculated with and without the test article with a fluorescence-labeled antibody, respectively: the cells inoculated with the test article show fluorescence characteristic of the virus and those not inoculated do not show it.

1.4.2.3.1.1.2 Serum neutralization test

Propagate the test article with appropriate cultured cells: virus-specific cytopathic changes shall be identified, and the propagation shall be neutralized by a specific antiserum.

1.4.2.3.1.2 Sterility test

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

1.4.2.3.1.3 Test for freedom from Mycoplasma contamination

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

1.4.2.3.1.4 Test for freedom from extraneous viruses

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

1.4.2.3.1.5 Test for stability confirmation of recombinant gene

If gene recombination technology is used for preparation of the master seed virus, the test given in the Test for Stability Confirmation of Recombinant Gene of the General Tests shall apply.

1.4.2.3.2 Working seed virus

1.4.2.3.2.1 Sterility test

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

1.4.2.3.2.2 Test for freedom from Mycoplasma contamination

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

1.4.2.3.3 Production seed virus

For storage, the following tests shall be performed:

1.4.2.3.3.1 Sterility test

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

1.4.2.3.3.2 Test for freedom from Mycoplasma contamination

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

1.4.2.4 Inactivated bacteria vaccine

1.4.2.4.1 Master seed bacteria

1.4.2.4.1.1 Identification test

The test for identification of bacteria species shall be performed in compliance with an appropriate morphological characterization test method, biochemical characterization test method, or other approved test methods.

1.4.2.4.1.2 Test for freedom from contaminant microorganisms

The test given in the respective sections of the parts of vaccine (seed lot product) shall apply. If no test method is given in the respective sections of the parts of vaccine (seed lot product), the test shall be performed in the approved manner.

1.4.2.4.1.3 Test for stability confirmation of recombinant gene

If gene recombination technology is used for preparation of the master seed bacteria, the test given in the Test for Stability Confirmation of Recombinant Gene of the General Tests shall apply.

1.4.2.4.2 Working seed bacteria

1.4.2.4.2.1 Test for freedom from contaminant microorganisms

The test given in the respective sections of the parts of vaccine (seed lot product) shall apply. If no test method is given in the respective sections of the parts of vaccine (seed lot product), the test shall be performed in the approved manner.

1.4.2.4.3 Production seed bacteria

For storage, the following tests shall be performed:

1.4.2.4.3.1 Test for freedom from contaminant microorganisms

The test given in the respective sections of the parts of vaccine (seed lot product) shall apply. If no test method is given in the respective sections of the parts of vaccine (seed lot product), the test shall be performed in the approved manner.

1.4.2.5 Recombinant protein vaccine

1.4.2.5.1 Expression system in recombinant virus

1.4.2.5.1.1 Master seed virus

1.4.2.5.1.1.1 Identification test

The test given in 1.4.2.1.1.1 shall apply.

1.4.2.5.1.1.2 Sterility test

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

1.4.2.5.1.1.3 Test for freedom from Mycoplasma contamination

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

1.4.2.5.1.1.4 Test for freedom from extraneous viruses

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

1.4.2.5.1.1.5 Test for stability confirmation of recombinant gene

The test given in the Test for Stability Confirmation of Recombinant Gene of the General Tests shall apply.

1.4.2.5.1.2 Working seed virus

1.4.2.5.1.2.1 Sterility test

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

1.4.2.5.1.2.2 Test for freedom from Mycoplasma contamination

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

1.4.2.5.1.3 Production seed virus

For storage, the following tests shall be performed:

1.4.2.5.1.3.1 Sterility test

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

1.4.2.5.1.3.2 Test for freedom from Mycoplasma contamination

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

1.4.2.5.2 Expression system in recombinant bacteria

1.4.2.5.2.1 Master seed bacteria

1.4.2.5.2.1.1 Identification test

The test for identification of bacteria species shall be performed in compliance with an appropriate morphological characterization test method, biochemical characterization test method, or other approved test methods.

1.4.2.5.2.1.2 Test for freedom from contaminant microorganisms

The test given in the respective sections of the parts of vaccine (seed lot product) shall apply. If no test method is given in the respective sections of the parts of vaccine (seed lot product), the test shall be performed in the approved manner.

1.4.2.5.2.1.3 Test for stability confirmation of recombinant gene

The test given in the Test for Stability Confirmation of Recombinant Gene of the General Tests shall apply.

1.4.2.5.2.2 Working seed bacteria

1.4.2.5.2.2.1 Test for freedom from contaminant microorganisms

The test given in the respective sections of the parts of vaccine (seed lot product) shall apply. If no test method is given in the respective sections of the parts of vaccine (seed lot product), the test shall be performed in the approved manner.

1.4.2.5.2.3 Production seed bacteria

For storage, the following tests shall be performed:

1.4.2.5.2.3.1 Test for freedom from contaminant microorganisms

The test given in the respective sections of the parts of vaccine (seed lot product) shall apply. If no test method is given in the respective sections of the parts of vaccine (seed lot product), the test shall be performed in the approved manner.

1.4.2.6 Live coccidia vaccine

1.4.2.6.1 Master seed coccidia

1.4.2.6.1.1 Identification test

The test for identification of coccidia shall be performed in compliance with an appropriate morphological characterization test method, PCR, enzyme electrophoresis, or other approved test methods.

1.4.2.6.1.2 Sterility test

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

1.4.2.6.1.3 Test for freedom from Mycoplasma contamination

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

1.4.2.6.1.4 Test for freedom from extraneous viruses

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

1.4.2.6.1.5 Target animal immunogenicity test

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

1.4.2.6.1.6 Target animal safety test

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

1.4.2.6.1.7 Test for absence of reversion to virulence

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

1.4.2.6.2 Working seed coccidia

1.4.2.6.2.1 Sterility test

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

1.4.2.6.2.2 Test for freedom from Mycoplasma contamination

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

1.4.2.6.3 Production seed coccidia

For storage, the following tests shall be performed:

1.4.2.6.3.1 Sterility test

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

1.4.2.6.3.2 Test for freedom from Mycoplasma contamination

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

2 Cell seeds

2.1 Cell line

2.1.1 Range of passage number

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 a three-fold increase in cell counts than an increase in the population doubling time shall be regarded as the passage of one generation. Note that this does not apply if otherwise approved by the Minister of Agriculture, Forestry and Fisheries based on the test results that assure their suitability as cells for production.

2.1.2 Preparation method

Cell suspension shall be dispensed in one continuous working session with a series of processes in accordance with the approved method to ensure their homogeneity and stability and prevent contamination.

2.1.3 Storage

Store under the approved conditions.

2.1.4 Origin, specifications and test methods of cell seeds

2.1.4.1 Records on origin

2.1.4.1.1 Origin

Record the name of animal and organ from which the cells are derived and backgrounds of establishment (passages, cloning, establisher, timing) to the extent possible.

2.1.4.1.2 Passage history

Record the passage history and cloning, etc. after established and after distributed (or purchased).

2.1.4.1.3 Culture medium

Record the culture media used for passage, propagation, and preservation.

2.1.4.2 Specifications and test methods

2.1.4.2.1 Master cell seeds

2.1.4.2.1.1 Test for confirmation of cell properties

When observed for microscopic findings, cell growth rate, acid production, morphological characteristics, and other characteristics from which the cells are considered normal as the cell lines, the cells shall comply with the test.

2.1.4.2.1.2 Test for identification of the animal species of the cell

When the test is performed by fluorescent antibody assay, the animal species shall be identical to “animal and organ from which the cells are derived” in 2.1.4.1.1 Origin.

2.1.4.2.1.3 Sterility test

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

2.1.4.2.1.4 Test for freedom from Mycoplasma contamination

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

2.1.4.2.1.5 Test for freedom from extraneous viruses

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

2.1.4.2.1.6 Test for karyological (chromosomal) characterization

The test given in either of the following sections shall apply:

2.1.4.2.1.6.1 Test on master cell seeds and their passaged cells

The following tests shall be performed on each of the master cell seeds and the maximum passaged cells.

In a test on chromosomes in at least 50 dividing cells, the modal number (modal chromosome number) in the maximum passaged cells shall be within ±15% of that in the master cell seeds. All indicator chromosomes present in the master cell seeds shall also be identified in the maximum passaged cells.

2.1.4.2.1.6.2 Test on passaged cells

When the cultured cells are tested as specified in 2.1.4.2.1.6.2.1, 2.1.4.2.1.6.2.2, 2.1.4.2.1.6.2.3, 2.1.4.2.1.6.2.4, and 2.1.4.2.1.6.2.5, there are no differences between all the four cultures of the cells that passaged at not less than the number of passages used for production and master cell seeds: the cultured cells shall comply with the test.

2.1.4.2.1.6.2.1 Polyploidy test

A total of more than 300 cells from four cultures of the cells shall be examined for polyploidy.

2.1.4.2.1.6.2.2 Aneuploidy test

A total of more than 100 cells from four cultures of the cells shall be examined for aneuploidy.

2.1.4.2.1.6.2.3 Morphology abnormalities test

A total of more than 100 cells from four cultures of the cells shall be examined for morphology abnormalities of chromosomes.

2.1.4.2.1.6.2.4 Chromosome cleavage test

A total of more than 100 cells from four cultures of the cells shall be examined for chromosome cleavage.

2.1.4.2.1.6.2.5 Karyotype analysis test

A karyotype analysis test shall be performed on one cell from one of four cultures of the cells.

2.1.4.2.1.7 Test for freedom from tumorigenicity

If findings show that malignant tumors are suspected to be induced in the animals targeted for inoculation of seed lot products using the cell lines, culture by passage the master cell seeds and passage at least four cultures of the cells used for production of seed lot products up to or more than the maximum number: these cultured cells shall comply with the following test.

Athymic mice (nu/nu), or immunosuppressed mice or hamsters shall be used for the test. At least five animals shall be given by subcutaneous injection with more than 2×106 cells, respectively, and observed for 28 days.

During the period, no animal shall show evidence of tumor formation. As control, at least five animals shall be similarly injected with more than 2×106 HeLa cells, which are known to form tumors. At the end of observation for 28 days, at least 80% of the animals shall show tumor formation.

2.1.4.2.2 Working cell seeds

2.1.4.2.2.1 Test for confirmation of cell properties

When observed for microscopic findings, cell growth rate, acid production, morphological characteristics, and other characteristics from which the cells are considered normal as the cell lines, the cells shall comply with the test.

2.1.4.2.2.2 Sterility test

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

2.1.4.2.2.3 Test for freedom from Mycoplasma contamination

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

2.1.4.2.3 Production cell seeds

For storage, the following tests shall be performed:

2.1.4.2.3.1 Test for confirmation of cell properties

When observed for microscopic findings, cell growth rate, acid production, morphological characteristics, and other characteristics from which the cells are considered normal as the cell lines, the cells shall comply with the test.

2.1.4.2.3.2 Sterility test

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

2.1.4.2.3.3 Test for freedom from Mycoplasma contamination

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

2.2 Primary cells

2.2.1 Use conditions

For production of seed lot products, cell lines shall be used. Note that this does not apply if primary cells compliant with 2.2.4 Specifications and test methods are available.

2.2.2 Range of passage number

To obtain the production primary cell seed, the cells prepared from the animals shall not be passaged more than 10 times. However, this does not apply if otherwise approved by the Minister of Agriculture, Forestry and Fisheries based on the test results that assure their suitability as cells for production.

2.2.3 Storage

Store under the approved conditions.

2.2.4 Specifications and test methods

2.2.4.1 Animals from which primary cells are collected

The animals shall comply with the SPF Animal Specifications.

2.2.4.2 Specifications and test methods for primary cells

2.2.4.2.1 Master primary cell seed

2.2.4.2.1.1 Test for confirmation of cell properties

When observed for microscopic findings, cell growth rate, acid production, morphological characteristics, and other characteristics from which the cells are considered normal as the primary cells, the cells shall comply with the test.

2.2.4.2.1.2 Sterility test

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

2.2.4.2.1.3 Test for freedom from Mycoplasma contamination

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

2.2.4.2.2 Working primary cell seed

2.2.4.2.2.1 Test for confirmation of cell properties

When observed for microscopic findings, cell growth rate, acid production, morphological characteristics, and other characteristics from which the cells are considered normal as the primary cells, the cells shall comply with the test.

2.2.4.2.2.2 Sterility test

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

2.2.4.2.2.3 Test for freedom from Mycoplasma contamination

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

2.2.4.2.3 Production primary cell seed

For storage, the following tests shall be performed:

2.2.4.2.3.1 Test for confirmation of cell properties

The test given in the culture observation of cells for production specified in the parts of vaccine (seed lot product) shall apply. If no test method is given in the respective sections of the parts of vaccine (seed lot product), the test shall be performed in the approved manner.

2.2.4.2.3.2 Sterility test

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

2.2.4.2.3.3 Test for freedom from Mycoplasma contamination

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

3 Embryonated eggs

3.1 Animals from which embryonated eggs are collected

The animals shall comply with the SPF Animal Specifications.3.2 Specifications and test methods for embryonated eggs

3.2.1 Observation of embryonic eggs

The control embryonated eggs shall be incubated and observed without being inoculated with vaccine seeds under the same conditions as the culture of vaccine seeds. No abnormalities shall be detected in the embryos.

4 Chickens

4.1 Chickens

Chickens used for the production of seed lot products shall be derived from SPF chicken flocks as specified in 1.1 in the Materials for Live Vaccine Production. However, this does not apply to Chicken anemia virus among the pathogens listed in Table 1 in the Materials for Live Vaccine Production, if the absence of the virus is confirmed in the intermediate manufacturing process of productions, including the bulk material.

4.2 Specifications and test methods for chickens

4.2.1 Growth test

The control chickens shall be bred and observed without being inoculated with vaccine seeds under the same conditions as the culture of vaccine seeds. No abnormalities shall be detected.

5 Other materials

The materials for use necessary to manufacture or maintain the seeds such as the media, digestive fluid and other materials shall be free from microorganisms and foreign matter.