

2.4.2. Cultured bovine testicular cell inoculation test

2.4.2.1. Test method

Use the subcultured bovine testicular cells.

Inoculate 2 mL of the sample to the cultured cells of 20 cm² or more per mL, incubate at 34 to 36°C for 5 days, and observe the presence or absence of CPE. Then, subculture the cells in 10 small test tubes, incubate for 5 days, and observe the presence or not of CPE.

Remove the medium, add 0.5 mL of the maintenance medium containing bovine virus diarrhea-mucosal disease virus of about 10⁵ TCID₅₀ in 1 mL, perform roll-tube culture at 34 to 36°C for 7 days, and observe the presence or not of CPE.

2.4.2.2. Judgment

Consider the sample to meet this test when no CPE is observed in the cultured cells before inoculation of bovine virus diarrhea-mucosal disease virus during the observation period and CPE is observed in the cultured cells after inoculation.

2.5. Dog-derived cell inoculation test

2.5.1. Cultured canine kidney cell inoculation test

2.5.1.1. Observation of cells

2.5.1.1.1. Test method

Use the primary or established canine kidney cells.

Inoculate 2 mL of the sample to the cultured cells of 20 cm² or more per mL, incubate at 36°C for 5 days, and observe the presence or not of CPE. Then, subculture to the next passage, incubate at 36°C for 10 days, and observe the presence or not of CPE.

2.5.1.1.2. Judgment

Consider the sample to meet this test when no CPE is observed in the cultured cells during the observation period.

2.5.1.2. Hemadsorption test

2.5.1.2.1. Test method

On the last day of observation in 2.5.1.1, remove the medium, stratify 0.1 vol% red blood cell suspension of guinea pig, allow to stand at 4°C for 60 minutes, and observe the presence or not of hemadsorption under microscopy.

2.5.1.2.2. Judgment

Consider the sample to meet this test when no hemadsorption is observed in the cultured cell.

2.6. Cat-derived cell inoculation test

2.6.1. Cultured feline kidney cell inoculation test

2.6.1.1. Observation of cells

2.6.1.1.1. Test method

Use the primary or established feline kidney cells.

Inoculate 2 mL of the sample to the cultured cells of 20 cm² or more per mL, incubate at 36°C for 5 days, and observe the presence or not of CPE. Then, subculture to the next passage, incubate at 36°C for 10 days, and observe the presence or not of CPE.

2.6.1.1.2. Judgment

Consider the sample to meet this test when no CPE is observed in the cultured cells during the observation period.

2.6.1.2. Canine parvovirus and feline panleukopenia virus negation test

2.6.1.2.1. Test method

To the culture medium on the last day of observation in 2.6.1.1. add an equivalent amount of a bovine serum albuminized borate buffered saline solution. In addition, add 0.5 vol% porcine red blood cell suspension prepared with the VAD 6.0 solution in an equivalent amount to this mixture, allow to stand at 4°C for 18 hours, and observe the presence or not of hemagglutination.

2.6.1.2.2. Judgment

Consider the sample to meet this test when no hemagglutination is observed.

2.7. Other animal-derived cell inoculation test

2.7.1. Cultured guinea pig kidney cell inoculation test

2.7.1.1. Observation of cells

2.7.1.1.1. Test method

Use the primary guinea pig kidney cells.

Inoculate 2 mL of the sample to the cultured cells of 20 cm² or more per mL, incubate at 37°C for 10 days, and observe the presence or not of CPE.

2.7.1.1.2. Judgment

Consider the sample to meet this test when no CPE is observed in the cultured cells during the observation period.

2.7.1.2. Hemadsorption test

2.7.1.2.1. Test method

On the last day of observation in 2.7.1.1, wash the surface of the cells twice with a phosphate buffered saline solution, divide the cultured cells into 3 groups, stratify 0.1 vol% red blood cell suspensions of guinea pig, goose and chicken within 7 days of age on each group, allow to stand at 4°C for 60 minutes and at 37°C for 30 minutes, and then observe the presence or not of hemadsorption under microscopy.

2.7.1.2.2. Judgment

Consider the sample to meet this test when no hemadsorption is observed in the cultured cell.

2.7.2. MA-104 cell inoculation test

2.7.2.1. Culture and observation

2.7.2.1.1. Test method

Use MA-104 cells.

Inoculate 0.1 mL each of the sample into 10 or more test tubes containing the cultured cells, allow to adsorb at 37°C for 60 minutes, wash the surface of cell with a phosphate buffered saline solution, add the medium containing an appropriate amount of trypsin, perform rolling culture at 37°C for 7 days, and observe the presence or not of CPE.

2.7.2.1.2. Judgment

Consider the sample to meet this test when no CPE is observed in the cultured cells during the observation period.

2.7.2.2. Rotavirus negation test

2.7.2.2.1. Test method

Inoculate 0.1 mL of the sample treated with trypsin, incubate at 37°C for 48 hours, perform the fluorescent antibody technique against rotavirus, and observe by the UV excitation mode.

2.7.2.2.2. Judgment

Consider the sample to meet this test when no specific fluorescent antigen is observed in the cultured cells.

2.8. Animal inoculation test

2.8.1. Bovine inoculation test

2.8.1.1. Bovine leukemia virus negation test

2.8.1.1.1. Test method

Use healthy cows weighing 100 to 200 kg.

Inject 10 mL of the sample to cow intramuscularly, the serum obtained from the blood collected at 2 and 3 months, and perform the agar gel precipitation reaction using the bovine leukemia virus agar gel precipitation reaction antigen.

2.8.1.1.2. Judgment

Consider the sample to meet this test when there is no precipitation line between the test serum and the antigen, which is fused together with the precipitation line observed between anti-bovine leukemia virus serum and the antigen.

2.8.2. Suckling mouse intracerebral inoculation test

2.8.2.1. Test method

Use mice within 3 days of age.

Inject 0.02 mL each of the sample intracerebrally to 10 mice, inject the supernatant obtained by centrifugation of the mixed emulsion of the brains collected on 5 days

intracerebrally to additional 10 mice, and observe for 10 days.

2.8.2.2. Judgment

Consider the sample to meet this test when no death or no neurological symptom is observed in mice.

Materials for manufacturing of poultry live vaccine

The growing eggs, the cultured cells and the bovine serum used as the materials for manufacturing of live vaccine shall meet the following specifications:

1. Growing eggs

1.1. Growing chicken eggs

The growing chicken eggs used for manufacturing of live vaccine shall originate from the SPF chicken group in which it was confirmed that there is no infection with the pathogens listed in Table 1 by the test and procedures shown in Table 1 or those considered to be equivalent.

1.2. Growing quail eggs

The growing quail eggs used for manufacturing of live vaccine shall originate from the SPF quail group in which it was confirmed that there is no infection with the pathogens listed in Table 2 by the test and procedures shown in Table 2 or those considered to be equivalent.

1.3. Growing duck eggs

The growing duck eggs used for manufacturing of live vaccine shall originate from the SPF duck group in which it was confirmed that there is no infection with the pathogens listed in Table 3 by the test and procedures shown in Table 3 or those considered to be equivalent.

2. Cultured cells

2.1. Cells originated from chicken embryo

2.1.1. Primary chicken embryonic cells

The primary chicken embryonic cells (chicken embryonic fibroblasts) used for manufacturing of live vaccine shall be prepared from the chicken embryos originated from the growing chicken eggs which met the specifications in 1.1.

2.1.2. Primary chicken embryonic kidney cells

The primary chicken embryonic kidney cells used for manufacturing of live vaccine shall be prepared from the chicken embryonic kidney originated from the growing chicken eggs which met the specifications in 1.1.

2.1.3. Primary chicken embryonic liver cells

The primary chicken embryonic liver cells used for manufacturing of live vaccine shall be prepared from the chicken embryonic liver originated from the growing chicken eggs which met the specifications in 1.1.

2.2. Cells originated from chickens

2.2.1. Primary chicken kidney cells

The primary chicken kidney cells used for manufacturing of live vaccine shall be prepared from the chicken kidney originated from the growing chicken eggs which met the specifications in 1.1.

2.3. Cells originated from quail embryos

2.3.1. Primary quail embryonic cells

The primary quail embryonic cells (quail embryonic fibroblasts) used for manufacturing of live vaccine shall be prepared from the quail embryos originated from the growing quail eggs which met the specifications in 1.2.

2.4. Cells originated from duck embryos

2.4.1. Primary duck embryonic cells

The primary duck embryonic cells (duck embryonic fibroblasts) used for manufacturing of live vaccine shall be prepared from the duck embryos originated from the duck eggs which met the specifications in 1.3.

2.5. Cells originated from ducks

2.5.1. Primary duck kidney cells

The primary duck kidney cells used for manufacturing of live vaccine shall be prepared from the duck kidney originated from the growing duck eggs which met the specifications in 1.3.

2.6. Cells originated from pigs

2.6.1. Primary porcine kidney cells

The primary porcine kidney cells used for manufacturing of live vaccine shall be prepared from the porcine kidney without any lesion, which was extracted from the pigs showing no abnormalities such as fever, on which health care was conducted for 7 days or more before sacrifice.

3. Bovine serum

The bovine serum used for manufacturing of live vaccine was separated from the fresh blood of healthy cattle or bovine fetuses, sterilized through a filter, dispensed and immobilized. Using this as the sample, perform the test according to the Sterility Tests 1, 2 and 3 and the Detection of Extraneous Viruses in the Biological Products 2.3.1 and 2.3.2 in the General Tests, Processes and Apparatus: It shall meet the tests.

Table 1. Testing and treatment of SPF chicken group

Pathogen	Antigen tested ¹⁾	Timing of test and the number of animals tested				Test method ²⁾	Treatment
		Repetition 1		On and after repetition 2			
		Timing	Number of animals(%)	Timing	Number of animals(%)		
Newcastle disease virus	Ishii strain	8 to 12 weeks old	20	Every 3 months	10	HI	All the animals in the positive group and the mating group ³⁾ were sacrificed
Avian infectious bronchitis virus	M-41 strain	''	''	''	''	SN or DID	''
Avian leukemia virus	Sub-A,B	''	''	''	''	SN	''
Avian encephalomyelitis virus	Van Roekel strain	''	''	''	''	DID	''
Avian nephritis virus	G-4260 strain	''	''	''	''	FA	''
Avian infectious laryngotracheitis virus	NS-175 strain	''	''	''	''	DID	''
Reticulosis virus	T strain	''	''	''	''	DID	''
Marek's disease virus	JM strain	''	''	''	''	DID	''
Infectious Fabricius' bursa disease virus	J1 strain	''	''	''	''	DID	''
Avian reovirus	Uchida strain	''	''	''	''	DID	''
Avian adenovirus	Ote strain	''	''	''	''	DID	''
EDS-76 virus	JPA-1 strain	''	''	''	''	HI	''
Avian influenza virus	5331 strain	''	''	''	''	DID	''
Avian paramyxovirus	Yucaipa	''	''	''	''	HI	''
Haemophilus paragallinarum type	221 strain	''	''	''	''	HI	''
Salmonella pullorum	9-25 strain	''	100	''	100	AGG	''
Mycoplasma gallisepticum	S6 strain	''	''	''	''	AGG	''
Mycoplasma synoviae	WVU-1853 strain	''	''	''	''	AGG	''
Salmonella (except for S. pullorum)		Every day	''	Every day	''	Clinical observation	Positive chickens were sacrificed
Fowlpox virus		''	''	''	''	Clinical observation	All the animals in the positive group and the mating group ³⁾ were sacrificed

Note: 1) HI: Hemagglutination inhibition reaction, SN: Serum neutralization test, FA: Fluorescent antibody technique, DID: Double immunodiffusion, AGG: Aggregation reaction

2) The mating group is the group which is not completely isolated from the positive group.

Table 2. Testing and treatment of SPF quail group

Pathogen	Antigen tested ¹⁾	Timing of test and the number of animals tested				Test method ²⁾	Treatment
		Repetition 1		On and after repetition 2			
		Timing	Number of animals(%)	Timing	Number of animals(%)		
Newcastle disease virus	Ishii strain	4to 6 weeks old	20	Every 3 months	10	HI	All the animals in the positive group and the mating group ³⁾ were sacrificed
Avian leukemia virus	Sub-A,B	//	//	//	//	SN	//
Avian encephalomyelitis virus	Van Roekel strain	//	//	//	//	DID	//
Avian infectious laryngotracheitis virus	NS-175 strain	//	//	//	//	FA	//
Reticulosis virus	T strain	//	//	//	//	DID	//
Marek's disease virus	JM strain	//	//	//	//	DID	//
Infectious Fabricius' bursa disease virus	J1 strain	//	//	//	//	DID	//
Avian reovirus	Uchida strain	//	//	//	//	DID	//
Avian adenovirus	Ote strain	//	//	//	//	DID	//
Avian influenza virus	5331 strain	//	//	//	//	DID	//
Avian paramyxovirus	Yucaipa	//	//	//	//	FA	//
Haemophilus paragallinarum type	221 strain	//	//	//	//	HI	//
Salmonella pullorum	9-25 strain	//	100	//	100	AGG	//
Mycoplasma gallisepticum	S6 strain	//	//	//	//	AGG	//
Mycoplasma synoviae	WVU-1853 strain	//	//	//	//	AGG	//
Salmonella (except for S. pullorum)		Every day	//	Every day	//	Clinical observation	//
Quailpox virus		//	//	//	//	Clinical observation	Positive quails were sacrificed

Note: 1) HI: Hemagglutination inhibition reaction, SN: Serum neutralization test, FA: Fluorescent antibody technique, DID: Double immunodiffusion, AGG: Aggregation reaction

2) The mating group is the group which is not completely isolated from the positive group.

Table 3. Testing and treatment of SPF duck group

Pathogen	Antigen tested ¹⁾	Timing of test and the number of animals tested				Test method ²⁾	Treatment
		Repetition 1		On and after repetition 2			
		Timing	Number of animals(%)	Timing	Number of animals(%)		
Newcastle disease virus	Ishii strain	10 to 12 weeks old	20	Every 3 months	10	HI	All the animals in the positive group and the mating group ³⁾ were sacrificed
Avian leukemia virus	Sub-A,B	"	"	"	"	SN	
Avian encephalomyelitis virus	Van Roekel strain	"	"	"	"	DID	
Reticulosis virus	T strain	"	"	"	"	DID	
Marek's disease virus	JM strain	"	"	"	"	DID	
Infectious Fabricius' bursa disease virus	J1 strain	"	"	"	"	DID	
Avian reovirus	Uchida strain	"	"	"	"	DID	
Avian adenovirus	Ote strain	"	"	"	"	DID	
EDS-76 virus	JPA-1 strain	"	"	"	"	HI	
Avian influenza virus	5331 strain	"	"	"	"	DID	
Duck hepatitis virus	Chiba strain	"	"	"	"	SN	
Avian paramyxovirus	Yucaipa	"	"	"	"	HI	
Haemophilus paragallinarum type	221 strain	"	"	"	"	HI	
Salmonella pullorum	9-25 strain	"	100	"	100	AGG	
Mycoplasma gallisepticum	S6 strain	"	"	"	"	AGG	
Mycoplasma synoviae	WVU-1853 strain	"	"	"	"	AGG	
Salmonella (except for S. pullorum)		Every day	"	Every day	"	Clinical observation	

Note: 1) HI: Hemagglutination inhibition reaction, SN: Serum neutralization test, FA: Fluorescent antibody technique, DID: Double immunodiffusion, AGG: Aggregation reaction

2) The mating group is the group which is not completely isolated from the positive group.

Original species virus for manufacturing of hog cholera live virus vaccine

1. Definition

It is an original species virus for manufacturing of the "live vaccine of hog cholera" , which is a virus solution obtained by proliferating the GPE- strain of attenuated hog cholera virus with the primary guinea pig kidney cells and by lyophilizing the culture medium.

2. Preparation

2.1. Strain for manufacturing

2.1.1. Name

GPE- strain of attenuated hog cholera virus

2.1.2. Origin

The original strain of the GPE- strain of attenuated hog cholera was prepared by subculturing the ALD strain of attenuated hog cholera virus in the primary porcine testicular cells, the primary bovine testicular cells and the primary guinea pig kidney cells.

2.1.3. Description

The original strain and the original species viruses show no pathogenicity after subcutaneous or intramuscular administration to the pigs which are negative against the hog cholera virus antibody. It proliferates in the primary porcine testicular cells but does not show the END phenomenon (E marker). And the proliferation at 30°C in the primary guinea pig kidney cells is higher than that at 40°C (T marker) and more than 100 times higher than that of virulent hog cholera virus (G marker).

2.1.4. Subculture and storage

The original strain and the original species viruses shall be subcultured in the primary guinea pig kidney cells, and the original species virus shall be manufactured within 3 passages from the original strain virus.

The original strain shall be lyophilized and stored at 4°C or lower, and the original species virus shall be frozen and stored at -70°C or lower.

2.2. Manufacturing materials

2.2.1. Cultured cells

Use the primary guinea pig kidney cells (supplement 1).

2.2.2. Culture medium

Use the medium for cell proliferation (supplement 2) and the medium for cell

maintenance (supplement 3).

2.3. Undiluted solution

2.3.1. Cell culture

Consider the cells treated once and cultured as the individually cultured cells. The cultured cells before inoculation of virus shall not show any abnormality.

On the individually cultured cells, perform the test in 3.1.

2.3.2. Culture of the original species virus

To the primary guinea pig kidney cells, add the medium for cell maintenance containing the original strain virus at $10^{3.0}$ TCID₅₀ or more in 1 mL. Mix the high titer virus solution obtained by subculturing 2 or 3 passages at 30°C at the intervals of 6 to 8 days, filter or centrifuge, use the resultant filtrate or the supernatant as the undiluted solution of the original strain virus and freeze at -70°C or lower.

2.3.3. Preparation of the original species virus

Dispense about a half of the undiluted solution of the original species virus into 50-mL dispensing containers in 30 mL each and freeze at -70°C or lower, to use as the original species virus.

On the original species virus, perform the test in 3.2.

2.3.4. Preparation of the live vaccine of hog cholera for the field test of the original species virus

Use about the remaining half of the undiluted solution of the original species virus as the undiluted solution of "live vaccine of hog cholera", and according to the preparation, manufacture the live vaccine of hog cholera for the field test of the original species vaccine for 50,000 animals or more (hereinafter referred to as the "test vaccine").

On the test vaccine, perform the test in 3.3.

3. Test method

3.1. Test of the cultured cells

Use more than 20% of the individually cultured cells as the control cultured cells, and on these cells, perform the following tests:

3.1.1. Incubation and observation

Incubate the control cultured cells without inoculation of virus under the same conditions as those for virus culture.

Pool and subculture the control cultured cells, incubate for 7 days and observe: The cultured cells shall not show any CPE.

3.1.2. Hemadsorption test

On the last day of observation in 3.1.1, remove the medium in the culture bottle, wash the cell surface with 2- portions of phosphate buffered saline solution, divide into 3 groups, stratify the 0.1 vol% red blood cell suspensions of guinea pigs, geese and the chickens within 7 days of age on the cell surface, allow to stand for 60 minutes, and observe the presence or absence of hemadsorption.

The cultured cells shall not show any hemadsorption.

3.1.3. Staining test of inclusion body

On the last day of observation in 3.1.1, wash the cultured cover slip with phosphate buffered saline solution, fix, stain with Giemsa and observe the presence or absence of inclusion body.

The cultured cells shall not show any inclusion body.

3.1.4. Aberrant virus negation test

Use the culture medium collected on the last day of observation in 3.1.1 as the sample and perform the test according to the Detection of Extraneous Viruses in the Biological Products in 2.3.1 and 2.7.1 in the

General Tests, Processes and Apparatus: It shall meet the test.

3.2. Tests of the original species virus

3.2.1. Characteristic test

Perform the test according to the Characteristic Test in the General Tests, Processes and Apparatus: It shall be a liquid or a dried product of unique color tone. The description in the dispensing container shall be uniform.

3.2.2. Sterility test

Perform the test according to the Sterility Tests 1, 2 and 3 in the General Tests, Processes and Apparatus: It shall meet the test.

3.2.3. Aberrant virus negation test

Perform the test according to the Detection of Extraneous Viruses in the Biological Products 1.1, 2.3.1.1, 2.3.1.2, 2.3.2, 2.4.2 and 2.7.1 in the General Tests, Processes and Apparatus: It shall meet the test.

Additionally, as the anti-hog cholera virus serum for neutralization, use the immobilized one described in the supplement 4.

3.2.4. Identification and virus content tests

3.2.4.1. Test materials

3.2.4.1.1. Sample

Dilute the test sample or the solution, which was obtained by dissolving the test

sample in 20 mL of the culture medium for maintenance of cells, 10-fold serially with the culture medium for cell proliferation, and use the dilution at each step as the sample.

3.2.4.1.2. Cultured cells

Use the primary porcine testicular cells or the subcultured porcine kidney cells.

3.2.4.2. Test methods

Use a 96-well tissue culture plate. Dispense 0.1 mL each of the sample into 10 wells in each row. Into 2 wells in each row, dispense 0.1 mL each of the culture medium for cell proliferation and use as the control cells. Into each well, dispense 0.1 mL each of the cell suspension prepared with the culture medium for cell proliferation. Perform stationary culture in a carbon dioxide incubator at 37°C for 5 to 7 days, remove the medium, wash with 2-portions of the rinsing solution (supplement 5) and fix.

Into each well of the fixed plate, dispense 0.05 mL each of the monoclonal antibody of anti-hog cholera virus-infected cells (supplement 7), which was diluted with the antibody diluent (supplement 6) to the optimum concentration, and react at 37°C for 60 minutes.

Wash with 4-portions of the rinsing solution, dispense 0.05 mL each of the peroxidase labeled anti-mouse immunoglobulin diluted with the antibody diluent to the optimum concentration into each well, and react at 37°C for 40 to 60 minutes.

Wash with 4-portions of the rinsing solution, dispense 0.1 mL each of the substrate solution (supplement 8) into each well, react at room temperature for 10 to 30 minutes, stop the reaction by adding 0.05 mL each of 2.5 mol/L sulfuric acid into each well, and determine the absorbance in each well at a wave length of 492/630 nm, respectively.

3.2.4.3. Judgment

Consider the well showing the absorbance value more than twice higher than the mean absorbance value of the control cells as the hog cholera virus-infected cells, and calculate the TCID₅₀.

The virus content of the original species virus shall be 10^{6.0} TCID₅₀ or higher in 1 mL.

3.2.5. Marker tests

3.2.5.1. E marker test

3.2.5.1.1. Test materials

3.2.5.1.1.1. Sample

Dilute the test sample or the solution, which was obtained by dissolving the test sample in 20 mL of the culture medium for maintenance of cells, and the subcultured ALD strain of hog cholera virus cells adjust to about $10^{4.0}$ TCID₅₀ per 1 mL to use as the samples.

3.2.5.1.1.2. Cultured cells

Use the primary porcine testicular cells.

3.2.5.1.1.3. Newcastle disease virus

Use the TCND strain or the Miyadera strain.

3.2.5.1.2. Test method

Dispense 0.1 mL each of the samples into more than 10 (well) each small test tubes, and add 0.5 mL each of the cultured cells suspended in the culture medium for cell proliferation. Perform stationary culture at 37°C for 4 days, remove the medium, add 0.5 mL each of the culture medium for maintenance of cells, containing Newcastle disease virus of about $10^{6.0}$ PFU, and incubate at 37°C for 3 days.

3.2.5.1.3. Judgment

The test sample or the solution obtained by dissolving the test sample in 20 mL of the culture medium for maintenance of cells shall not shown any CPE, but the ALD strain shall show CPE.

3.2.5.2. T and G marker tests

3.2.5.2.1. Test materials

3.2.5.2.1.1. Sample

Dilute the test sample or the solution, which was obtained by dissolving the test sample in 20 mL of the culture medium for maintenance of cells, with the culture medium for maintenance of cells, and use the virus solution containing $10^{3.0}$ TCID₅₀ in 1 mL as the samples.

3.2.5.2.1.2. Cultured cells

Use the primary guinea pig kidney cells.

3.2.5.2.2. Test method

Incubate the cultured cells in a small test tube to make a monolayer. Inoculate 0.1 mL each of the sample on more than 20 (well) cultured cells, divide into 2 groups, and perform stationary culture at 30°C and 40°C for 6 to 8 days. Collect the culture medium for every group, mix, and determine the virus content according to 3.2.4.

3.2.5.2.3. Judgment

Proliferation at 30°C shall exceed that at 40°C (T marker), and the virus content at 30°C shall be $10^{4.5}$ TCID₅₀ or more in 1 mL (G marker).

3.2.6. Safety test

3.2.6.1. Test materials

3.2.6.1.1. Materials for injection

Use the test sample or the solution, which was obtained by dissolving the test sample in 20 mL of the culture medium for maintenance of cells, as the materials for injection.

3.2.6.1.2. Test animals

Use the pigs weighing 20 to 40 kg.

3.2.6.2. Test method

Administer 10 mL each of the materials for injection subcutaneously or intramuscularly to 4 test animals and perform clinical observation for 2 weeks.

3.2.6.3. Judgment

During the period of observation, the test animals shall not show any clinical abnormality.

3.2.7. Titer test

3.2.7.1. Test materials

3.2.7.1.1. Materials for injection

The test sample was diluted with the culture medium for maintenance of cells, and adjusted to the virus solution containing $10^{3.0}$ TCID₅₀ in 1 mL as the materials for injection.

3.2.7.1.2. Test animals

Use the pigs weighing 20 to 40 kg.

3.2.7.2. Test method

Use 4 test animals as the test group and 2 as the control group. Administer 1 mL each of the materials for injection subcutaneously or intramuscularly to the test group, 2 weeks later, administer 10^5 lethal dose of the ALD strain of hog cholera virus subcutaneously to both groups, and observe for 3 weeks.

3.2.7.3. Judgment

During the period of observation, the test group shall not show any abnormality, but the control group shall show the symptoms of hog cholera within 7 days and die during the period of observation.

3.3. Test of the test vaccine

3.3.1. Self-examination

Perform the test according to the tests on subplot and lot in the "live vaccine of hog cholera": It shall meet all the tests.

3.3.2. National examination

The test vaccine shall undergo the examination based on the Article 43 of the Pharmaceutical Affairs Law (hereinafter referred to as the "national examination") as the "live vaccine of hog cholera" and pass the test.

3.3.3. Field examination

3.3.3.1. Test materials

Use the test vaccine and the vaccine manufactured from the original species virus manufactured at 1 lot before the original species virus concerned (hereinafter referred to as the "reference vaccine").

3.3.3.2. Testing facility

Two or more pig farms in the outdoors, from which precise and objective clinical trial data can be obtained.

3.3.3.3. Test animals

Use more than 400 healthy pigs kept at the pig farms in 3.3.3.2, whose dams are known and to whom the live vaccine of hog cholera has not been inoculated.

3.3.3.4. Test method

3.3.3.4.1. Safety test

Divide one each litter of pigs into 2 groups of almost similar number, subcutaneously or intramuscularly administer the test vaccine to one group and the reference vaccine to the other group, and perform clinical observation for 2 weeks.

3.3.3.4.2. Potency test

From the pigs used in 3.3.3.4.1, collect the blood at injection of vaccine and at 2 months after injection, and determine the neutralizing antibody titer against hog cholera virus in each serum. Determine the neutralizing antibody titer using the ALD strain of hog cholera virus and the TCND strain or the Miyadera strain of Newcastle disease virus as the challenge virus by the END method.

3.3.3.5. Judgment

3.3.3.5.1. Safety test

During the period of observation, no significant difference shall be observed in the incidence of abnormal pigs between both groups.

3.3.3.5.2. Potency test

No significant difference shall be observed in the antibody titer between both groups.

4. Storage and expiry date

Store the frozen product at -70°C or lower.

The expiry date shall be 5 years later.

Supplement 1: Guinea pigs

Guinea pigs for manufacturing shall not be infected with parainfluenza type I virus (HVJ) and Japanese encephalitis virus and shall be clinically healthy.

Supplement 2: Culture medium for cell proliferation

In 1,000 ml:

Tryptose phosphate broth	2.95g
or Lactoalbumin	5g
Bovine or goat serum	100 mL
Eagle's MEM or Earle's solution	q.s.

Adjust the pH to 6.8 to 7.2 with sodium bicarbonate.

Use the bovine or goat serum which is negative against the neutralizing antibody against bovine viral diarrhea-mucosal disease virus.

The minimum amount of antibiotics may be added.

Supplement 3: Culture medium for maintenance of cells

In 1,000 ml:

Tryptose phosphate broth	2.95g
or Lactoalbumin	5g
Bovine or goat serum	50 mL
Eagle's MEM or Earle's solution	q.s.

Adjust the pH to 6.8 to 7.2 with sodium bicarbonate.

Use the bovine or goat serum which is negative against the neutralizing antibody against bovine viral diarrhea-mucosal disease virus.

The minimum amount of antibiotics may be added.

Supplement 4: Anti-hog cholera virus serum

Rabbit or goat serum immunized with the ALD strain of hog cholera virus or the strain considered as appropriate, whose neutralizing antibody titer is 1,000 times or higher.

Supplement 5: Rinsing solution

Mixture of the solutions A and B.

In 800 mL of the solution A:

Sodium chloride	8.0g
Potassium chloride	0.2g
Anhydrous disodium hydrogen phosphate	1.15g
Anhydrous potassium dihydrogen phosphate	0.2g
Water	q.s.

In 200 mL of the solution B:

Anhydrous calcium chloride	0.1g
Magnesium chloride (hexahydrate)	0.1g
Water	q.s.

Supplement 6: Antibody diluent

Dissolve bovine serum albumin fraction V in Hanks' solution or the supplement 4 so as to be 0.5 to 1.0 g/dL.

Supplement 7: Monoclonal antibody of anti-hog cholera virus-infected cells

Distributed by the Testing Laboratory on the Drugs for Animals.

Supplement 8: Substrate solution

To 50 mL of 0.2 mol/L phosphate-0.1 mol/L citrate buffer solution (pH 5.0), add 25 mg of orthophenylenediamine and 0.01 mL of hydrogen peroxide solution.

Prepare before use.

Detection of extraneous viruses in the live viral vaccine for mammals

Live viral vaccine + Immune sera to vaccine strains

(Final product) ↓

(One dose) 37°C for 60 min.

Inoculated to ↓

Cells derived
from animals
for which
vaccines
intended

↓

Incubate at 37°C
for 5 days

Detection of specified viruses

(Specified in the monograph)

↓

Passage

Incubate at 37°C
for 10 days.

Judgement

No CPE and/or no HAD

For example

Canine distemper vaccine + anti-canine distemper serum

↓
37°C 60min.

↓
Inoculate to

↓
MDCK cells

↓
CRFK cells

(canine parvovirus negation)

↓
37°C 5days

↓
37°C 5days

↓
Passage

↓
Passage

↓
37°C 10days

↓
37°C 10days

↓
No CPE and
no HAD with
guinea-pig RBC

↓
No CPE and no HA of
culture fluid with
porcine RBC

Suckling mouse intracerebral inoculation test

(rabies virus negation)

Inoculate intracerebrally to 10 mice (within 3 days)

↓
Inoculate the mixed emulsion of the brains

Collected on 5 days to additional 10 mice (within 3 days).

Observed for 10 days.

↓
No death or no neurological symptom.