**Newcastle Disease diagnostic Hemagglutination Antigen**

1 Definition

Newcastle Disease diagnostic Hemagglutination Antigen is antigen for hemagglutination inhibition that is prepared from an inactivated suspension of Newcastle disease virus that is propagated in embryonated chicken eggs.

2 Production methods

2.1 Virus strain used for production

2.1.1 Name

Newcastle disease virus Ishii strain or strain approved as equivalent thereof

2.1.2 Properties

This virus strain propagates in the allantoic cavity of embryonated chicken eggs aged 9 to 11 days, and the allantoic fluid shows chicken hemagglutination ability.

2.1.3 Passage and storage

The original strain and seed virus shall be passaged in embryonated chicken eggs as specified in 1.1 in the Materials for Live Vaccine Production.

The original strain shall not be passaged more than three times, and the seed virus no more than twice.

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

2.2 Materials for production

2.2.1 Embryonated chicken egg

Embryonated chicken eggs aged 9 to 11 days as specified in 1.1 in the Materials for Live Vaccine Production shall be used.

2.3 Bulk material

2.3.1 Virus cultivation

The seed virus shall be inoculated into the allantoic cavity of embryonated chicken eggs and incubated for three to five days. The supernatant obtained by centrifuging the harvested allantoic fluid shall serve as the virus suspension.

2.3.2 Inactivation

Equal volumes of diethyl ether shall be added to the virus suspension, which is then reacted at 37°C, centrifuged, and the aqueous layer collected. To this layer, an equal volume of 1/25 mol/L potassium periodate shall be added, reacted at 37°C for 60 minutes, followed by the addition of an equal volume of 10 w/v% glucose solution to serve as the bulk material.

The bulk material shall be tested as specified in 3.1.

2.4 Final bulk

The bulk material shall be mixed, and this mixture shall 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 3.2.

3 Test methods

3.1 Tests on bulk material

3.1.1 Inactivation test

3.1.1.1 Materials

The test article, embryonated chicken eggs aged 9 to 11 days specified in 1.1 in the Materials for Live Vaccine Production, and chicken red blood cell suspension adjusted at a concentration of 0.5 vol% after mixing the sampled blood from at least two chickens aged 6 to 18 weeks and washing three times with physiological saline shall be used.

3.1.1.2 Test procedures

A 0.1 mL portion of the test article shall be injected into the allantoic cavity of each of five embryonated chicken eggs and incubated at 37°C for five days, and the allantoic fluid shall be collected. Following a further passaging using the same procedure, an equal volume of chicken red blood cell suspension shall be added to each allantoic fluid, and a hemagglutination test shall be performed.

3.1.1.3 Judgment

No agglutination of chicken red blood cells shall be detected in the allantoic fluid.

3.1.2 Specificity test

3.1.2.1 Materials

The test article, reference antigen (Note 1), reference positive serum (Note 2), reference negative serum (Note 3), and chicken red blood cell suspension as specified in 3.1.1.1 shall be used.

3.1.2.2 Test procedures

The test article and the reference antigen shall be adjusted the concentrations to 4 units per 0.2 mL. Three each of the reference positive serum and the reference negative serum shall be diluted five-fold and then serially diluted two-fold. Equal volumes of antigen solution shall be added to 0.2 mL of each of these diluted sera and mixed. After reacting for 10 minutes, 0.4 mL of chicken red blood cell suspension shall be added to each and then mixed by agitating. The mixture shall then be allowed to stand for 60 minutes for judgment.

3.1.2.3 Judgment

The reference positive sera, when mixed with the test article and reference antigen, shall demonstrate the specified hemagglutination inhibition antibody titer, and the antibody titer of the reference negative serum must be less than ten-fold.

3.1.3 Potency test

3.1.3.1 Materials

The test article and the reference antigen dissolved in 1 mL of physiological saline and chicken red blood cell suspension specified in 3.1.1.1 shall be used.

3.1.3.2 Test procedures

The test article and the reference antigen shall be diluted ten-fold with physiological saline and serially diluted two-fold to serve as the test materials.

Equal volumes of chicken red blood cell suspension shall be added to 0.4 mL of each of the test materials and mixed by agitating. The mixture shall then be allowed to stand for 60 minutes for judgment.

3.1.3.3 Judgment

When the highest dilution of the test article and reference antigen that result in complete hemagglutination is defined as the antigen titer, the antigen titer of the test article shall be 640 -fold or higher. In this case, the reference antigen must show the specified antigen titer.

3.2 Tests on final product

Physiological saline shall be used as the dissolving solution.

3.2.1 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.

3.2.2 Vacuum degree test

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

3.2.3 Test for moisture content

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

3.2.4 Specificity test

The test given in 3.1.2 shall apply.

3.2.5 Potency test

When the test is performed as specified in 3.1.3, the antigen titer of the test sample shall be 640 -fold or higher.

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.

5. Other

5.1 Indicated positive serum

Indicated positive serum that clearly indicates the antibody titer shall be attached.

Note 1 Reference antigen

Antigen distributed by National Veterinary Assay Laboratory

Note 2 Reference positive serum

Serum distributed by National Veterinary Assay Laboratory. It is obtained from chickens derived from 1.1 in the Materials for Live Vaccine Production that were immunized with Newcastle disease virus. It is dispensed into 1 mL portions and freeze-dried.

When hemagglutination inhibition test is performed using the reference antigens, the antibody titer shall be between 160 to 320-fold, 40 to 80-fold, and 10 to 20-fold.

Note 3 Reference negative serum

Chicken serum derived from 1.1 in the Materials for Live Vaccine Production. It is dispensed into 1 mL portions and freeze-dried.

When hemagglutination inhibition test is performed using the reference antigen, the antibody titer shall be less than ten-fold.

**Mycoplasma gallisepticum Rapid diagnostic Antigen**

1 Definition

Mycoplasma gallisepticum Rapid diagnostic Antigen is antigen for rapid agglutination that consists of a concentrated suspension of inactivated *Mycoplasma gallisepticum* supplemented with crystal violet.

2 Production methods

2.1 Bacterial strain used for production

2.1.1 Name

*Mycoplasma gallisepticum* S6 strain or strain approved as equivalent thereof

2.1.2 Passage and storage

The original strain and seed bacteria shall be passaged in a medium for Mycoplasma (Note 1) or a medium approved as suitable.

The original strain shall not be passaged more than three times, and the seed bacteria no more than five times unless otherwise specified by the Minister of Agriculture, Forestry and Fisheries.

The original strain and seed bacteria shall be freeze-dried and stored at 5°C or lower.

2.2 Materials for production

2.2.1 Medium

A medium for Mycoplasma or a medium approved as suitable for production shall be used.

2.3 Bulk material

2.3.1 Cultured bacterial medium

Seed bacteria shall be transferred to a medium and allowed to develop sufficiently through 2 to 3 passages at 37°C. These shall be further transferred to a medium and incubated at 37°C for 3 to 7 days. The resulting medium shall serve as the cultured bacterial medium.

The cultured bacterial medium shall be tested as specified in 3.1.

2.3.2 Bacterial harvesting

The culture bacterial medium shall be centrifuged, and resulting sediment of bacterial cells shall be resuspended in phosphate-buffered saline to make a concentrated bacterial solution. This solution shall then be stand at 2-5°C overnight or longer.

2.3.3 Concentration adjustment and addition of chemical compounds

The concentrated bacterial solution shall be adjusted with phosphate-buffered saline so that the concentration shall be 25 times that of McFarland turbidity standard No. 1. To this preparation, thiomersal and crystal violet shall be added at 0.01w/v% each. The mixture shall then be allowed to stand for at least one week at 2°C to 5°C for inactivation and staining, to serve as the bulk material.

The bulk material shall be tested as specified in 3.2.

2.4 Final bulk

The bulk materials shall be mixed and this mixture 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 3.3.

3 Test methods

3.1 Tests on cultured bacterial medium

3.1.1 Test for freedom from contaminant microorganisms

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

3.2 Tests on bulk material

3.2.1 Specificity test

3.2.1.1 Materials

The test article, anti-*Mycoplasma gallisepticum* rabbit serum (Note 2) and anti-*Mycoplasma synoviae* rabbit serum (Note 3) shall be used.

3.2.1.2 Test procedures

To one drop (approximately 0.03 mL) each of anti-*Mycoplasma gallisepticum* rabbit serum and anti-*Mycoplasma synoviae* rabbit serum, one drop (approximately 0.03 mL) of the test article shall be added and mixed thoroughly on a slide glass maintained at 20 to 25°C to examine the presence of agglutination.

3.2.1.3 Judgment

Agglutination must occur within one minute in the anti-*Mycoplasma gallisepticum* rabbit serum, while it should not occur within two minutes in the anti-*Mycoplasma synoviae* rabbit serum.

3.2.2 Potency test

3.2.2.1 Materials

The test article, reference antigen (Note 4), serum and blood from at least five *Mycoplasma gallisepticum* antibody positive chickens (Note 5), as well as serum and blood from at least five *Mycoplasma gallisepticum* antibody negative chickens (Note 6), shall be used.

3.2.2.2 Test procedures

Sera to be used as the material;

One drop (approximately 0.03 mL) each of the test article and reference antigen and one drop (approximately 0.03 mL) of serum shall be mixed thoroughly on a slide glass to examine the presence of agglutination.

Blood to be used as the material；

Two drops (approximately 0.06 mL) each of the test article and reference antigen and one drop (approximately 0.03 mL) of blood shall be added and mixed thoroughly on a slide glass maintained at 20°C to 25°C to examine the presence of agglutination.

3.2.2.3 Judgment

For positive chickens, agglutination must occur in both serum and blood within one minute, and for negative chickens, agglutination should not occur in both serum and blood within two minutes.

The appearance time for agglutination must generally coincide both the test article and the reference antigen.

3.3 Tests on final product

3.3.1 Properties test

When the test is performed as specified in the Properties Test of the General Tests, the final product shall be 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.

3.3.2 Specificity test

The test given in 3.2.1 shall apply.

3.3.3 Potency test

The test given in 3.2.2 shall apply.

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.

5. Others

5.1 Notes for descriptions in package inserts

1 Tests should be conducted in an environment constantly at 20°C to 25°C, and locations prone to direct sunlight and dust outside should be avoided.

2 When using a serum as a material, it should be fresh, and serum that has been frozen or stored for a long period should not be used.

3 The antigen should be occasionally shaken well both before and during use to ensure its homogeneity.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Note 1 | Medium for Mycoplasma |  | |  |
|  | 1,000 mL consists of the following: |  | |  |
|  | Basal medium for Mycoplasma\* | 17.3 g | | |
|  | Horse serum | 120 mL | | |
|  | Benzylpenicillin potassium | 250,000 to 500,000 units | | |
|  | Water | | Residual quantity | |

Adjust the pH to 7.7 - 7.9.

Dissolve the basal medium for Mycoplasma in water with the aid of heat and autoclave at 121°C for 15 minutes. After cooling, add filter-sterilized horse serum and benzylpenicillin potassium, and add sterile water to make 1,000 mL; alternatively, sterilize by filtration after mixing all these materials.

\*Basal medium for Mycoplasma

|  |  |  |
| --- | --- | --- |
|  | Dried bovine heart infusion (equivalent to 100 mL) | 1.3 g |
|  | Peptone | 10 g |
|  | Sodium chloride | 5 g |
|  | Glucose | 1 g |
|  | Thallous acetate | 0.25 g |

Note 2 Anti-*Mycoplasma gallisepticum* rabbit serum

Serum obtained from a rabbit immunized with *Mycoplasma gallisepticum*, dispensed into 1 mL portions and freeze-dried. When tube agglutination is performed *in vitro* using the reference antigen, the agglutination titer shall be 20-fold or higher.

Note 3 Anti-*Mycoplasma synoviae* rabbit serum

Serum obtained from a rabbit immunized with *Mycoplasma synoviae*, dispensed into 1 mL portions and freeze-dried. When tube agglutination is performed *in vitro* using the reference antigen provided in Mycoplasma synoviae Rapid diagnostic Antigen, the agglutination titer shall be 20-fold or higher.

Note 4 Reference antigen

“Mycoplasma gallisepticum Rapid diagnostic Antigen” or other antigens recognized as equivalent by the National Veterinary Assay Laboratory

Note 5 *Mycoplasma gallisepticum* antibody positive chickens

Chickens derived from 1.1 in the Materials for Live Vaccine Production and infected with *Mycoplasma gallisepticum*. When tube agglutination is performed *in vitro* using the reference antigen, the serum agglutination titer shall be 20-fold or higher.

Note 6 *Mycoplasma gallisepticum* antibody negative chickens

Chickens derived from 1.1 in the Materials for Live Vaccine Production. When tube agglutination is performed *in vitro* using the reference antigen, the serum agglutination titer shall be less than five-fold.