

INFLUENZA HA VACCINE

1. Descriptive definition

“Influenza HA Vaccine” is a clear or slightly whitish turbid liquid product containing hemagglutinin (hereafter referred to as "HA" in this monograph) of influenza virus (hereafter referred to as "virus" in this monograph).

2. Production control

2.1 Source materials

2.1.1 Strains of virus used for production

Strains of virus of types A and B specified elsewhere shall be used.

2.1.2 Embryonated hens' eggs used for production

Hens' eggs incubated for 10-12 days (hereafter referred to as "eggs" in this monograph) shall be used.

2.2 Bulk material

2.2.1 Virus suspension

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

2.2.2 Purification and fractionation

Virus shall be concentrated and purified by appropriate procedures and disintegrated by ether or other appropriate agents. After rapid removal of lipolytic materials, the suspension of HA fraction shall be collected. Formaldehyde or other appropriate stabilizer shall be added to the suspension to serve as the single-strain bulk material.

The single bulk material shall be subjected to the tests given in 3.1.

2.3 Final bulk

The single-strain bulk material shall be diluted with buffered physiological saline or other suitable medium and mixed to make the potency given in 3.2.10. Appropriate stabilizer and/or preservatives may be used.

3. Control tests

3.1 Tests on single-strain bulk material

3.1.1 Staining test

The test given in General Tests shall apply.

3.1.2 Fractionation test

The test given in 3.2.2 shall apply.

3.1.3 Sterility test

The test given in General Tests shall apply.

3.1.4 Pyrogen test

The test given in General Tests shall apply to a test sample prepared by diluting with physiological saline to make the virus content no lower than one-third that of the final bulk. One milliliter of the test sample per kg body weight shall be subjected to the test given in General Tests.

3.1.5 Test for leukopenic toxicity

The test sample shall be prepared by dilution, if necessary, with physiological saline to make the concentration equal to that of the final bulk. The test method given in 3.2.9 shall apply.

3.2 Tests on final product

Following tests shall apply to each final lot.

3.2.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 8.0.

3.2.2 Fractionation test

The linear density gradient of sucrose from 20 to 50% in 4.8 mL shall be made in centrifugal tubes of 1/2 inch in diameter and 2 inches in length. For preparation of the gradient, 20% and 50 % sucrose solutions shall be used.

The test sample shall be diluted in the 20% sucrose solution to contain either about 300 CCA/mL upon determination by the modified Miller-Stanley's method or about 30 µg/mL HA protein.

Each gradient shall be overlaid with 0.2 mL of the dilution and centrifuged with $100,000 \times g$ at a maximal centrifugal radius for 90 minutes at $4 \pm 1^\circ\text{C}$ in a rotor of the swinging bucket type.

Immediately after centrifugation, the content of the tube shall be fractionated into portions of 0.25 mL, and each portion shall be determined for the hemagglutination value as well as the density of sucrose. The results shall indicate the disintegration of virus. Either the accumulated hemagglutination values of the upper 2.5-mL portion and the lower 2.5-mL portion or hemagglutination values of the pooled upper portion and the lower portion, respectively, shall be determined. The value of the upper portion shall be higher than that of the lower portion.

If there is any doubtful result in the above measurements, the disintegration of virus shall be demonstrated by electron-microscopic examination to judge the acceptability.

3.2.3 Test for freedom from ether

If ether is used as a lipolytic agent, the test sample shall have no residual odor of ether.

3.2.4 Test for protein content

When the method given in General Tests is applied, the protein content shall be no higher than 240 µg/mL.

3.2.5 Test for thimerosal content

If thimerosal is used as a preservative, the thimerosal content shall be no higher than 0.012 w/v%, when the test given in General Tests for thimerosal content is applied.

3.2.6 Test for formaldehyde content

When the test given in General Tests is applied, the formaldehyde content shall be no higher than 0.01 w/v%.

3.2.7 Sterility test

The test given in General Tests shall apply.

3.2.8 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.2.9 Test for leukopenic toxicity

3.2.9.1 Materials

Test sample and Reference Influenza Vaccine for the test for mouse leucocytes-decreasing toxicity (hereafter referred to as "Toxicity Reference" in this monograph) shall be used. Physiological saline shall be used for diluting the Toxicity Reference.

3.2.9.2 Test procedure

The Toxicity Reference shall be diluted with physiological saline to make at least three levels of appropriate logarithmic serial dilutions. Each dilution of the Toxicity Reference and test sample shall be given by intraperitoneal injection at a dose of 0.5 mL into at least 5 mice aged 4 weeks. Physiological saline shall be injected into at least 10 mice to serve as the control in the same manner.

The leukocyte counting on peripheral blood shall be conducted 12–18 hours after injection.

3.2.9.3 Criterion for judgment

The leukopenic toxicity in mouse of the test sample calculated relative to that of the Toxicity Reference shall be no higher than the value corresponding to 80% of the leukocyte count of the control in reference to that of the Toxicity Reference upon statistical analysis.

3.2.10 Potency test

The Single-Radial-Immunodiffusion test or Immunogenicity test shall apply.

3.2.10.1 Single-Radial-Immunodiffusion test

3.2.10.1.1 Materials

The test sample, Reference Influenza HA Antigen (for Single-Radial-Immunodiffusion test) (hereafter referred to as "Reference Antigen" in this monograph) and agarose plates with a proper amount of Reference Anti-influenza HA Antiserum against each strain of virus contained in the product (hereafter referred to as "SRD plate" in this monograph) shall be used.

3.2.10.1.2 Test procedures

The test sample and Reference Antigen shall be solubilized with a suitable detergent. The test sample and Reference Antigen shall be diluted in proper dilutions by using Dulbecco's phosphate-buffered saline (pH 7.4) containing 0.05 w/v% sodium azide. An appropriate volume of the diluted sample and Reference Antigen shall be added to the well of an SRD plate. The SRD plate shall be kept in a humidified container at 20–25°C for more than 18 hours, washed with water, dried and stained. The diameter of stained zones surrounding antigen wells shall be measured in 2 directions at right angles.

3.2.10.1.3 Criterion for judgment

The potency of the test sample shall be no less than 15 µg of HA of each virus per 0.5 mL upon statistical analysis.

3.2.10.2 Immunogenicity test

Potency shall be determined by titrating the neutralizing antibody produced in the immunized mice in egg as a substrate.

3.2.10.2.1 Materials

The test sample, Reference Influenza HA Vaccine (for neutralization test in eggs) (hereafter referred to as "Reference" in this monograph) and infected allantoic fluid of eggs with each strain of virus contained in the product (hereafter referred to as "challenge virus suspension") shall be used. The challenge virus suspension shall contain 10^3 – 10^6 EID₅₀ of virus per 0.1 mL. The diluent shall be the nutrient broth.

3.2.10.2.2 Test procedures

The test sample and Reference shall be properly diluted in five-fold serial dilutions. Each dilution shall be given by intraperitoneal injection at a dose of 0.5 mL into at least 10 mice aged 4 weeks. Approximately equal amounts of blood shall be taken from the animals of each group 21 days after immunizing injection and pooled to obtain serum. Each pooled serum shall be diluted two-fold. Equal volumes of each dilution and the challenge virus suspension shall be mixed and kept at $34 \pm 1^\circ\text{C}$ for 60 minutes. Each mixture shall be inoculated at a dose of 0.2 mL into the allantoic cavities of at least 5 eggs per dose. The inoculated eggs shall be incubated at $34 \pm 1^\circ\text{C}$ for 2 days and kept standing at 2 – 5°C overnight. Individual allantoic fluids shall be tested for agglutinability on chicken red blood cells.

Each challenge virus suspension shall be serially diluted and inoculated into eggs to confirm the virus infectivity titer (EID₅₀) within the specified range.

3.2.10.2.3 Criterion for judgment

The potency of the test sample shall be no less than that of the Reference upon statistical analysis.

3.2.11 Inactivation test

The test sample shall be inoculated at a dose of 0.2 mL into the allantoic cavities of at least 6 eggs. The inoculated eggs shall be incubated at $34 \pm 1^\circ$ for 3 days. The allantoic fluid shall be harvested and pooled. The pool shall be similarly inoculated and the inoculated eggs shall be incubated. The allantoic fluid of each egg shall be tested for agglutinability on chicken red blood cells.

No fluid shall show positive hemagglutination. In case there are positive results, equal portions from the positive fluids shall be pooled. The pool shall be inoculated at a dose of 0.2 mL into the allantoic cavities of at least 6 eggs and the inoculated eggs shall be incubated at $34 \pm 1^\circ$ for 3 days. The allantoic fluid of each egg shall then be tested for agglutinability on chicken red blood cells. If no fluid shows positive results in the repeat test, the test sample shall be judged as acceptable.

3.2.12 Test for toxicity to mouse weight gain

The test sample shall be given by intraperitoneal injection into at least 5 mice aged 4 weeks at a dose of 0.5 mL. The body weight shall be recorded before and about 24 hours after injection. The mean body weight after the injection shall be no less than that before injection when the results are compared statistically.

3.2.13 Identity test

The test shall be conducted by agglutination of chicken red blood cells.

4. Storage and expiry date

The expiry date shall be one year.

5. Other requirements

5.1 Modification of the proper name

Monotypic or single strain products may be manufactured, if necessary. In such a case, the name of the type or of the strain of the virus shall be added to the proper name.

5.2 Labeling

Names of the strains of virus used as the source of HA contained in the product and their contents per mL.

5.3 Information to be provided in package insert and other labeling

- (1) The method used for disintegration of virus
- (2) Recommended human dose and route of administration, as follows:

A dose or 2 doses of 0.5 mL are given by subcutaneous injection at a 1–4 weeks interval, with the dose changed to 0.1 mL for those aged below 1 year, 0.2 mL for those aged 1–5 years, and 0.3 mL for those aged 6–12 years.

In case there are differences in recommendation, the dose and/or route of administration shall be modified.