

**MINIMUM REQUIREMENTS  
FOR  
BIOLOGICAL PRODUCTS**

**National Institute of Infectious Diseases**

**Japan, 2006**

# MINIMUM REQUIREMENTS FOR BIOLOGICAL PRODUCTS

**Notice:** This English version of the Minimum Requirements for Biological Products is prepared for the convenience of users unfamiliar with the Japanese language. When and if any discrepancy arises between the Japanese original and its English translation, the former is authentic.

National Institute of Infectious Diseases  
Japan, 2006

## Foreword

The “Minimum Requirements for Biological Products” was developed to specify the manufacturing methods, descriptive definitions, quality, storage, test methods, etc. of vaccines, antitoxins, blood products and other biological products for human use according to Article 42(1) of the Pharmaceutical Affairs Law, which states, “the Minister of Health, Labour and Welfare is authorized to establish the necessary standards for the manufacturing methods, descriptive definitions, quality, storage, etc. of drugs that require special cautions for use from the points of health and hygiene after hearing the opinions of the Pharmaceutical Affairs and Food Sanitation Council.” The Minimum Requirements for Biological Products had not been revised since 1993; however, an update had been urged because of recent scientific and technological advancement, development of new test methods, and increasing public concern about the safety of products prepared from animal or other biological materials. The update was also considered necessary to maintain consistency between the Minimum Requirements for Biological Products and such other official requirements as the Pharmaceutical Affairs Law, the Standards for Biological Materials (Ministry Ordinance No. 210 issued in 2003), the Japanese Pharmacopoeia, and World Health Organization (WHO) requirements. Thus, the Ministry of Health, Labour and Welfare established the “Expert Committee for Revision of the Minimum Requirements for Biological Products” to gather advice, recommendations, and ideas regarding revision, and prepared this updated guideline (Ministry Ordinance No. 155) in March 2004, with the intent of providing a standards guideline to meet the latest level of science and technology and the current needs of society.

In 1947, the National Institute of Health (currently the National Institute of Infectious Diseases) was established as a national agency to manage and control the quality of biological preparations. The quality control system for vaccines and other biological materials was initially introduced under the guidance of the General Headquarters of the Supreme Commander for the Allied Powers. Since its establishment, the institute has continued to provide safer and more effective vaccines, strengthening collaborations with the National Control Laboratories (NCLs) of other countries and the WHO. In just the past 20 years, the institute has contributed to the manufacture and quality control of vaccines in Asian countries and other regions by accepting trainees and sending experts as part of the Official Development Assistance (ODA) program. It is my sincere hope that this publication will benefit those activities.

March 1, 2006

Takeshi Kurata, M.D., Ph.D.  
Director-General  
National Institute of Infectious Diseases

## CONTENTS

|   |          |
|---|----------|
| <b>GENERAL RULES</b> .....  | <b>7</b> |
| Influenza Vaccine .....   | 13       |
| Influenza HA Vaccine.....   | 17       |
| Freeze-dried Inactivated Tissue Culture Hepatitis A Vaccine.....  | 22       |
| Freeze-dried Live Attenuated Mumps Vaccine.....   | 25       |
| Gas Gangrene Antitoxin, Equine.....   | 30       |
| Freeze-dried Gas Gangrene Antitoxin, Equine.....  | 33       |
| Inactivated Rabies Vaccine.....   | 35       |
| Freeze-dried Inactivated Tissue Culture Rabies Vaccine.....   | 40       |
| Cholera Vaccine.....  | 44       |
| Freeze-dried Diphtheria Antitoxin, Equine .....   | 47       |
| Diphtheria Toxoid.....  | 50       |
| Adsorbed Diphtheria Toxoid .....  | 54       |
| Adsorbed Diphtheria Toxoid for Adult Use .....  | 56       |
| Diphtheria-tetanus Combined Toxoid .....  | 57       |
| Adsorbed Diphtheria-tetanus Combined Toxoid .....   | 59       |
| Varicella Antigen .....   | 61       |
| Freeze-dried Live Attenuated Varicella Vaccine.....   | 66       |
| Typhoid-paratyphoid Vaccine.....  | 72       |
| Purified Tuberculin.....  | 75       |
| Smallpox Vaccine.....   | 80       |
| Freeze-dried Smallpox Vaccine.....  | 84       |
| Smallpox Vaccine Prepared in Cell Culture .....   | 86       |
| Freeze-dried Smallpox Vaccine Prepared in Cell Culture .....  | 90       |
| Japanese Encephalitis Vaccine .....   | 92       |
| Freeze-dried Japanese Encephalitis Vaccine .....  | 96       |
| Pneumococcal Vaccine Polyvalent.....  | 98       |
| Tetanus Toxoid.....   | 107      |
| Adsorbed Tetanus Toxoid.....  | 110      |
| Freeze-dried Habu Antivenom, Equine .....   | 112      |
| Adsorbed Habu-venom Toxoid .....  | 116      |
| Adsorbed Hepatitis B Vaccine.....   | 119      |
| Adsorbed Hepatitis B Vaccine (Prepared from huGK-14 Cells) .....  | 124      |
| Recombinant Adsorbed Hepatitis B Vaccine (Prepared from Yeast).....   | 127      |
| Recombinant Adsorbed Hepatitis B Vaccine (Prepared from Chinese Hamster Ovary Cells) .....                    | 130      |
| Recombinant Adsorbed Hepatitis B Vaccine Containing pre-S2 Antigen and HBs Antigen (Prepared from Yeast)..... | 133      |
| Freeze-dried BCG for Intravesical Use (Connaught Strain).....   | 136      |
| Freeze-dried BCG for Intravesical Use (Japanese Strain).....  | 140      |
| Freeze-dried BCG Vaccine (for Percutaneous Use) .....   | 143      |
| Pertussis Vaccine .....   | 146      |
| Adsorbed Purified Pertussis Vaccine.....  | 150      |

|  |     |
|--|-----|
| Diphtheria-pertussis Combined Vaccine .....                                  | 154 |
| Diphtheria-pertussis-tetanus Combined Vaccine.....                           | 157 |
| Adsorbed Diphtheria-purified Pertussis-tetanus Combined Vaccine.....         | 160 |
| Freeze-dried Live Attenuated Rubella Vaccine .....                           | 165 |
| Typhus Vaccine .....   | 171 |
| Freeze-dried Botulism Antitoxin, Equine.....                                 | 174 |
| Live Oral Poliomyelitis Vaccine.....   | 177 |
| Freeze-dried Live Attenuated Measles Vaccine.....                            | 183 |
| Freeze-dried Live Attenuated Measles, Mumps, Rubella Combined Vaccine.....   | 188 |
| Freeze-dried Live Attenuated Measles, Rubella Combined Vaccine.....          | 191 |
| Freeze-dried Mamushi Antivenom, Equine.....                                  | 194 |
| Weil's Disease and Akiyami Combined Vaccine .....                            | 198 |
| Whole Human Blood.....   | 202 |
| Concentrated Human Red Blood Cells.....                                      | 205 |
| Washed Human Red Blood Cells .....   | 206 |
| Leukocyte-poor Red Blood Cells .....   | 207 |
| Frozen-thawed Human Red Blood Cells.....                                     | 209 |
| Fresh-frozen Human Plasma .....  | 211 |
| Platelet Concentrate.....  | 213 |
| Human Plasma Protein Fraction.....   | 215 |
| Human Serum Albumin.....   | 217 |
| Freeze-dried Human Fibrinogen .....  | 219 |
| Freeze-dried Human Blood Coagulation Factor VIII.....                        | 221 |
| Freeze-dried Concentrated Blood Coagulation Factor VIII .....                | 223 |
| Freeze-dried Human Blood Coagulation Factor IX Complex .....                 | 225 |
| Freeze-dried Concentrated Human Blood Coagulation Factor IX .....            | 227 |
| Normal Human Immunoglobulin .....  | 229 |
| Reduced Alkylated Normal Human Immunoglobulin.....                           | 231 |
| Freeze-dried Ion-exchange-resin-treated Normal Human Immunoglobulin .....    | 233 |
| Freeze-dried Sulfonated Normal Human Immunoglobulin.....                     | 235 |
| pH 4-treated Normal Human Immunoglobulin .....                               | 237 |
| Freeze-dried pH 4-treated Normal Human Immunoglobulin .....                  | 239 |
| Freeze-dried Plasmin-treated Normal Human Immunoglobulin .....               | 241 |
| Freeze-dried Pepsin-treated Normal Human Immunoglobulin .....                | 243 |
| Polyethylene Glycol-treated Normal Human Immunoglobulin.....                 | 245 |
| Freeze-dried Polyethylene Glycol-treated Normal Human Immunoglobulin .....   | 247 |
| Human Anti-HBs Immunoglobulin .....  | 249 |
| Freeze-dried Human Anti-HBs Immunoglobulin.....                              | 251 |
| Polyethylene Glycol-treated Human Anti-HBs Immunoglobulin .....              | 253 |
| Freeze-dried Polyethylene Glycol-treated Human Anti-HBs Immunoglobulin ..... | 255 |
| Human Anti-D(Rho) Immunoglobulin .....                                       | 257 |
| Freeze-dried Human Anti-D(Rho) Immunoglobulin.....                           | 259 |
| Human Antl-tetanus Immunoglobulin.....                                       | 261 |
| Freeze-dried Human Antl-tetanus Immunoglobulin.....                          | 263 |
| Polyethylene Glycol-treated Human Antl-tetanus Immunoglobulin .....          | 265 |

|  |            |
|--|------------|
| Freeze-dried Polyethylene Glycol-treated Human Anti-tetanus Immunoglobulin ..... | 267        |
| Freeze-dried Human Antithrombin III Concentrate .....                            | 269        |
| Freeze-dried Human Activated Protein C Concentrate .....                         | 271        |
| Human Haptoglobin .....  | 273        |
| <b>GENERAL TESTS .....</b>   | <b>275</b> |
| A. Test Procedures .....   | 275        |
| Test for Aluminum Content .....  | 275        |
| Test for Freedom from Abnormal Toxicity (General Safety Tests) .....             | 276        |
| Test for Chloride Content .....  | 277        |
| Bacterial Endotoxins Test .....  | 278        |
| Test for Potassium Content .....   | 279        |
| Test for Moisture Content .....  | 280        |
| Test for Citric Acid Content .....   | 281        |
| Test for Sodium Citrate Content .....  | 282        |
| Test for Mycobacterial Sterility .....   | 284        |
| Test for Optical Density .....   | 285        |
| Test for Measuring the Potency of Human Anti-HBs Immunoglobulin .....            | 286        |
| Test for Measuring the Potency of Anti-D antibody .....                          | 287        |
| Test for Freedom from Anti-complement Activity .....                             | 288        |
| Test for Weight Variation .....  | 289        |
| Cellulose Acetate Membrane Electrophoretic Test .....                            | 290        |
| Staining Test .....  | 291        |
| Test for Protein Content .....   | 292        |
| Test for Protein Nitrogen Content .....  | 293        |
| Test for Thimerosal Content .....  | 294        |
| Test for Sugar Content .....   | 296        |
| Test for Sodium Content .....  | 298        |
| Test for Heat Stability .....  | 299        |
| Test for Tetanus Antitoxin Titer Determination .....                             | 300        |
| Pyrogen Test .....   | 301        |
| Test for pH .....  | 303        |
| Test for Phenol Content .....  | 304        |
| Test for Heme Content .....  | 305        |
| Test for Hemoglobin Content .....  | 306        |
| Test for Formaldehyde Content .....  | 307        |
| Mycoplasma Test .....  | 308        |
| Antibody Assay against Measles Virus .....                                       | 310        |
| Sterility Test .....   | 312        |
| Test for Sodium Dihydrogenphosphate Content .....                                | 313        |
| B. Standards, Reference Preparations, Test Toxins and Units .....                | 314        |
| C. Reagents, Test Solutions, etc. ....   | 322        |
| D. Buffered Solutions and Culture Media .....                                    | 337        |

## General Rules

1. These standards specify manufacturing methods, properties, quality, storage, and other matters for biological products listed in the monograph of drugs (hereafter referred to as “monographs”). These standards are abbreviated to “biological standards” in this document.
2. In addition to the General Rules of these biological standards, the Biological Ingredients Standards (Notification No. 210, Ministry of Health, Labour and Welfare, 2003) and the Second General Rules for Blood Products (hereafter referred to as “Blood Product General Rules”) are applicable to “whole human blood” and all the subsequent blood products listed.
3. The Japanese Pharmacopoeia is specified by the Pharmaceutical Affairs Law (Law No. 145, 1960), and the Japanese Industrial Standards are specified by the Industrial Standardization Law (Law No. 185, 1949).
4. “Standard names” imply names or alias names listed in the respective articles for drugs. Standard names are considered nonproprietary names when applied to Article 50 of the Pharmaceutical Affairs Law.
5. Drugs are to be tested according to the provisions given in the pertinent monograph, General Rules, the General Rules, Blood Product General Rules, and the provisions of General Tests.
6. Standard names enclosed in single quotation marks (‘ ’) indicate that the properties and quality conform to those specified in these standards, and those enclosed in double quotation marks (“ ”) indicate substances that exhibit specific biological activities.
7. As a rule, symbols used in these standards are those specified in the JP. As an exception, gravity “g” is used for gravity acceleration.
8. The following abbreviations are used as main units of bioassay:
  - BWDU: Body weight-decreasing unit
  - CCA: Chicken red cell agglutination
  - CCID: Cell culture-infective dose
  - CFU: Colony-forming unit
  - EID: Egg-infective dose
  - FFU: Focus-forming unit
  - HSU: Histamine-sensitizing unit
  - IU: International unit

LT: Limes Tod dose  
LD: Lethal dose  
Lf: Limit of flocculation  
LPU: Leukocyte-promoting unit  
LR: Limes reaction  
MRD: Minimum reaction dose  
PFU: Plaque-forming unit/pock-forming unit  
U: Unit

9. Units used to represent titers in monographs are determined by comparing corresponding standard preparations, if available. In addition, when WHO international standard preparations are available, a reference preparation with the same titer as the international unit is set and considered as 1 unit.

10. The Celsius scale is used for temperature measurement and “°C” is added after Arabic numerals. In addition, temperature regulations shall comply with the General Rules of JP.

11. Solutions without solvent name indicate water solutions.

12. Unless otherwise specified, appropriate solvents including suspension liquids (hereafter “solvents” include suspension liquids) shall be attached to freeze-dried drug preparations. In addition, when the label instruction states “Add solvent” only, the attached solvent shall be added to the drug preparation according to the method stated on the container or other labeling materials.

13. Unless otherwise specified, chemical agents that are listed in JP and used to manufacture drugs in monographs shall comply with JP requirements, whereas those not listed in JP but specified in the Japanese Industrial Standards shall comply with their specifications depending on the purpose of use.

14. Inactivating and stabilizer specified by the term, for example, “use suitable agents” in the monograph of drugs shall be safe for use at the usual amount used and shall not inhibit the beneficial effects of the drug or interfere with pharmaceutical tests.

15. The containers are the devices which hold drugs. The concept of a container also includes component parts such as the stopper or cap.

Unless otherwise specified, hermetic containers used for drugs in monographs and attached solvents shall comply with the specifications of either the Glass Container Tests for Injections, JP, Plastic Container Tests, JP, or Rubber Closure Tests for Aqueous Infusions, JP. Hermetic containers shall be light-resistant.

Hermetic containers are those specified in the General Rules of JP.

Light-resistant containers protect the contents from the transmission of light that might affect the properties and quality specified for individual drug products during routine handling, transportation, and storage.

16. The actual amount of drugs in monographs and attached solvents shall be slightly greater than the labeled amount and sufficient for the administration of the labeled dose. Unless otherwise specified, the extent of excess in individual and average amounts of liquid drugs, when they constitute a lot, shall comply with JP's General Rules for Injections. In addition, unless otherwise specified, actual weights of solid drugs that constitute a lot shall comply with the Weight Variation Test of the General Tests.

17. "Seed lots" indicate homogeneous suspensions of specific viruses, bacteria, and cells obtained from single cultures, dispensed to vials, and stored properly to maintain their genetic properties.

18. "Final bulk" indicates a bulk drug prepared in one vessel and ready for immediate filling into final containers. The content shall be completely homogeneous in terms of pharmaceutical properties and qualities. Shaking is allowed to maintain homogeneity.

19. "Final lot" indicates the products filled into small containers from a final bulk and sealed, and if necessary, freeze-dried and hermetically sealed.

20. "Lot" indicates a group of "final lot" having uniform character and quality that has been filled in one working session from a "final bulk" within specified limits.

21. In general, the same manufacturing number is assigned to the products of one lot. However, additional numbers or marks shall be assigned to the individual final lots, if manufactured under different operating conditions.

22. Generally, tests on specifications for final lot products required in monographs are collectively performed with the sample from the same manufacturing number. However, these tests may be omitted when such products are categorized under the Article 21 above and if the quality of the products is assured to be homogeneous and to comply with the Minimum Requirements for Biological Products on the basis of records of process control, production-process validation, and quality control tests.

23. In regard to "Descriptive definition," the term "white" means white or near-white, and "colorless" means colorless or near-colorless.

In regard to the tests for color, unless otherwise specified, final lot products in immediate containers shall be examined against a white background.

In regard to the tests for clarity, unless otherwise specified, final lot products in immediate containers shall be examined against a white or black background.

24. Unless otherwise specified, as a rule, drugs in monographs and their attached solvents shall comply with the Foreign Insoluble Matter Test for Injections in the General Tests of JP. Blood products for transfusion are excluded from this requirement.

25. Tests on specifications for monographs with solvents, except for moisture content or tests specified separately, shall be conducted using solutions or suspensions prepared with the solvents attached according to the method indicated on their immediate containers for final lot products. The amount of solvent used shall be accurately measured and carefully added, especially when used for physiochemical tests. Tests on monographs stored in the frozen condition shall be conducted after thawing at an appropriate temperature.

26. The “Inactivation Test” evaluates the loss or reduction in biological activity of viable microorganisms used in the production of a drug below the level specified in the requirements.

The “Detoxication Test” evaluates the loss of toxicity of toxic substances present during the production process below the level specified in the requirements.

The “( *name of microorganism or substance*) Test” determines the absence of substances or microorganisms indicated in ( ) below the level specified in the requirements.

The “Label Check Test” judges the authenticity of the product according to the label on the container and is generally conducted after the printing of information on the immediate container is completed.

27. Unless otherwise specified, tests shall be conducted at room temperature.

28. Unless otherwise specified, tests shall be conducted with “B: Standards, References, Test Toxins, and Units”, “C: Reagents, Test Solutions, and Others”, and “D: Buffers and Media” in the General Tests. The term “water” for use in the tests indicates purified water specified by JP.

29. To “accurately weigh” means weighing to the specified digit.

To “exactly measure” means measuring the specified whole volume using pipettes, measuring flasks, or burettes.

To “precisely measure” means weighing to the lowest digit, e.g., 0.1, 0.01, or 0.001mg.

30. “Approximately” in collecting the desired amount or quantity of, for example, samples indicates a variation in numerical value within  $\pm 10\%$ .

31. Usually, numerical values such as a quantity of inoculated sample shall indicate the specified value with a  $\pm 5\%$  variation.

32. When a test result is judged by comparing a specified value (hereafter referred to as “specified

value”) with actually measured values obtained from a test (hereafter referred to as “experimental values”), as a rule, experimental values shall be obtained to one digit lower than that for the specified value and rounded off to the same digit with the specified value.

33. Animals used in tests must be healthy. If animals exhibit incidental abnormalities, the test shall not meet the test requirements unless the abnormalities can be demonstrated to be unrelated to the pharmaceutical product tested.

34. Any test method may be employed as an alternative to the method specified by the Minimum Requirements for Biological Products if it is equally or more precise and accurate than the specified method. If test results are questionable, the final judgment of the test shall be made in accordance with the specified method of the Minimum Requirements for Biological Products.

35. The details of the biological test methods may be changed insofar as such changes do not affect the essential qualities of the test.

36. Unless otherwise specified or separately specified and officially approved, storage temperature shall be  $\leq 10^{\circ}\text{C}$ . However, liquid drug products shall not be frozen.

37. Unless otherwise specified, the expiry date for drug products that are designated to conform with national certification tests according to Article 43 (1) of the Pharmaceutical Affairs Law shall be set based on the date when the products pass required certification tests. The expiry date for other products shall be set based on the date when the products pass in-house tests.

38. The expiry date shall be specified in each monograph. When the approved expiry date differs from the specified date, the approved date may be employed.

39. “Delivery from warehouse” means the shipping of drug products from the storage of the manufacturing facility for sales or transport. Drug products shall be stored at a constant temperature before shipping.

40. “Dosage and administration” is the basis of establishing specifications for drug products and indicates the general usage of a product.

41. Entries on the immediate container for monographs required by Article 50 (6) of the Pharmaceutical Affairs Law shall be those listed below. These entries may be omitted when the volume of an ampoule or direct container is not more than 10 mL by providing them on the outer container or outer package instead.

(1) Storage conditions

(2) Expiry date (date and hour of expiry, when expiry date is set by hour)

- (3) Availability of an attached solvent for a drug product, with the solvent and method of preparation of the solvent (label on the product container) and solvent name, volume, composition, and quantity (label on the solvent container)
  - (4) Information required to be labeled in the monograph of the drug product
42. Package insert-related entries on the immediate container for monographs required by Article 52 (3) of the Pharmaceutical Affairs Law shall be those listed below:
- (1) Contraindications, if any
  - (2) Names and quantities of preservatives and stabilizers, if used
  - (3) Entries specified as “information to be included in the package insert, etc.” in the monograph of the drug product
43. Unless otherwise specified, animals used for the production of monographs or substances must be healthy.
44. Tests for sterility and pyrogenicity for blood products consisting of a lot shall be conducted for final lot products and at certain manufacturing processes as shown in the monographs. These tests may be omitted when sterility is assured from the records of process control, production-process validation, and quality control tests.
- Blood products not constituting a lot shall be sampled by the procedure specified in each monograph and tested for sterility. The products used in the test shall not be used in the manufacture of monographs or as raw materials for manufacture. Such products may be used as raw materials if sterility is not required in the manufacture or if sterility is confirmed after the sterility test is conducted.
45. In these Minimum Requirements for Biological Products, “equipment for transfusion” indicates equipment proven appropriate for transfusion of blood products such as whole human blood, ready for use, and disposable after single use.

# INFLUENZA VACCINE

## 1. Descriptive definition

“Influenza Vaccine” is a slightly whitish turbid liquid product containing inactivated 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 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 "egg" 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 incubated. Allantoic fluid containing propagated virus shall be harvested and pooled to serve as the single-strain virus suspension.

#### 2.2.2 Purification and inactivation

Virus shall be concentrated and purified by appropriate procedures. Virus shall then be inactivated by the addition of formalin or other suitable methods. The preparation containing purified and inactivated virus shall serve as the single-strain bulk material.

The single-strain 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 serve as the concentration specified elsewhere for each virus strain. Appropriate preservatives and stabilizer may be added.

## 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 Sterility test

The test given in General Tests shall apply.

#### 3.1.3 Inactivation test

The test given in 3.2.6 shall apply.

#### 3.1.4 Pyrogen test

The test given in General Tests shall apply to a test sample prepared by dilution with physiological saline to make the virus content no lower than one-sixth that of the final bulk. If the

result is not negative, whereas the same test repeated on the sample heated at 70°C for 30 minutes gives a negative result, the test sample shall be judged as acceptable.

#### 3.1.5 Test for leukopenic toxicity

The test sample shall be prepared by dilution, if necessary, with physiological saline to make the virus content twice that of the final bulk. Appropriate serial dilutions of the test sample shall be made. Each dilution 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 in the same manner into at least 10 mice to serve as the control.

Leukocyte counting of peripheral blood shall be conducted 12–18 hours after injection. The mean leukocyte count of each group shall be calculated. The virus content causing a 50% decrease shall be no lower than one-fourth that of the final bulk upon statistical analysis.

#### 3.1.6 Test for virus content

The 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 Test for protein content

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

#### 3.2.3 Test for thimerosal content

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

#### 3.2.4 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%.

#### 3.2.5 Sterility test

The test given in General Tests shall apply.

#### 3.2.6 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  $34 \pm 1^\circ\text{C}$  for 3 days. The allantoic fluid shall be harvested and pooled. The pool shall be similarly inoculated and the inoculated eggs incubated.

The allantoic fluid of each egg shall be tested for agglutinability on chicken red blood cells. No fluid shall show positive hemagglutination.

#### 3.2.7 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.2.8 Test for leukopenic toxicity

A two-fold dilution of the test sample shall be given by intraperitoneal injection at a dose of 0.5mL into at least 5 mice aged 4 weeks. The diluent shall be physiological saline.

Leukocytes in peripheral blood shall be counted before and 12–18 hours after injection.

The mean count after injection shall be no less than one-half that before injection upon statistical analysis.

The method using a control as given in 3.1.5 may be applied.

### 3.2.9 Test for virus content

The modified Miller-Stanley's method shall be applied to the test sample with Standard Influenza Vaccine (for CCA test) as the Standard. The CCA value of the test sample shall be within a range specified elsewhere. The test sample and the Standard shall be diluted in 0.01 mol/L phosphate-buffered sodium chloride solution (pH 7.0–7.2).

### 3.2.10 Potency test

Either the single radial immunodiffusion test or immunogenicity test shall be applied as a potency test.

#### 3.2.10.1 Single radial immunodiffusion test

##### 3.2.10.1.1 Materials

The test sample, Reference Influenza HA Antigen (for the single radial immunodiffusion test) (hereafter referred to as "Reference Antigen" in this monograph) and agarose plates with the 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 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 Vaccine (for neutralization test in eggs) (hereafter referred to as "Reference" in this monograph), and allantoic fluid of eggs infected with strains of virus contained in the product (hereafter referred to as "challenge virus suspension" in this monograph) shall be used. The challenge virus suspension shall contain  $10^3$ – $10^6$  EID<sub>50</sub> 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 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 age 5 weeks weighing

the same weight within a deviation of  $\pm 1$ g. Approximately equal amounts of blood shall be taken from the animals of each group 14 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  $2-5^\circ\text{C}$  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 ( $\text{EID}_{50}$ ) 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 when determined upon statistical analysis.

#### 3.2.11 Test for toxicity to mouse weight gain

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

#### 3.2.12 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 cases, the name of the type or strain of virus contained in the product shall be added to the proper name.

#### 5.2 Labeling

The name of the strains of viruses contained in the product and their contents in CCA value or HA protein content per mL shall be provided on the label.

#### 5.3 Information to be provided in package insert and other labeling

- (1) Method used for the inactivation of virus
- (2) The recommended human dose and route of administration, as follows:

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

# 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  $10^3$ – $10^6$  EID<sub>50</sub> 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  $2$ – $5^\circ\text{C}$  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 (EID<sub>50</sub>) 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.

# **FREEZE-DRIED INACTIVATED TISSUE CULTURE HEPATITIS A VACCINE**

## **1. Descriptive definition**

“Freeze-dried Inactivated Hepatitis A Vaccine” is a freeze-dried product containing inactivated hepatitis A virus (hereafter referred to as “virus” in this monograph). When reconstituted, it becomes a colorless clear liquid.

## **2. Production control**

### 2.1 Source materials

#### 2.1.1 Strains of virus used for production

Strains of virus approved as suitable for production shall be used. Passage of virus contained in the product shall be conducted under approved conditions and the designated number of passages shall not be exceeded.

#### 2.1.2 Strains of cells used for production

Strains of cell cultures approved as suitable for production shall be used. Passage of the cell cultures shall be conducted under approved culture conditions and the designated number of passages shall not be exceeded.

#### 2.1.3 Culture media

The cell culture medium can be supplemented with appropriate cell growth-promoting substances, phenol red at a concentration not higher than 0.002 w/v%, and minimal amounts of antibiotics, except penicillin.

### 2.2 Bulk material

#### 2.2.1 Cell culture

The cell culture shall be recovered from the frozen cell bank for production, and the designated number of passages of the cell culture shall not be exceeded. The cell culture shall be subjected to the test given in 3.1.

#### 2.2.2 Purified virus suspension

Virus shall be purified from infected cultured cells by appropriate procedures. The purified virus suspension shall be subjected to the test given in 3.2.

#### 2.2.3 Inactivation

Virus shall be inactivated by the addition of formalin. The purified virus suspension after inactivation shall serve as the bulk material. The bulk material shall be subjected to the test given in 3.3.

### 2.3 Final bulk and freeze-drying

The bulk material shall be diluted with buffered physiological saline or other suitable medium to serve as the final bulk. Appropriate stabilizer may be added. The final bulk shall be dispensed into final containers and freeze-dried.

## **3. Control tests**

### 3.1 Test on cell cultures

A quantity corresponding to more than 500 mL shall be used as the control cell culture. No cytopathic change shall be detected in control cell cultures incubating under the same culture conditions employed for the production of virus. During the observation period, cultures discarded due to any nonspecific or incidental reason shall be less than 20% of total control cell cultures.

### 3.2 Test on purified virus suspension

#### 3.2.1 Sterility test

Sterility Test and Mycoplasma Test given in General Tests shall apply.

#### 3.2.2 Test for purity

When the high-pressure liquid chromatography method is applied, the content of HAV antigen shall be no less than 98% of total protein.

#### 3.2.3 Test for cellular DNA content

When the dot blot hybridization test is applied under conditions in which 10 pg of cellular DNA can be detected, and using the DNA of the cell for production employed as the probe, the content of cellular DNA shall be no higher than 10 pg per 0.5 µg of HAV antigen.

### 3.3 Tests on bulk material

#### 3.3.1 Sterility test

The test given in General Tests shall apply.

#### 3.3.2 Inactivation test

A test sample corresponding to more than 100 doses in total shall be inoculated into 6 cell cultures in 75 cm<sup>2</sup> bottles, and incubated for 2 to 3 weeks. With 10 mL of the culture medium, the cells shall be freeze-thawed, sonicated, and centrifuged. The supernatant after centrifugation is regarded as the first passage material. The first passage material shall be inoculated into fresh cell cultures, and the samples obtained following the same process are regarded as the second passage material. No infectious virus shall be detected in the first- and second-passaged materials when subject to the immunofocus method and enzyme immunoassay.

### 3.4 Tests on final product

Following tests shall apply to each final lot.

#### 3.4.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### 3.4.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

#### 3.4.3 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%.

#### 3.4.4 Sterility test

The test given in General Tests shall apply.

#### 3.4.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.4.6 Test for antigen content

When the quantity test of HAV antigen is applied using enzyme immunoassay, the HAV antigen content shall be within the range of 0.7 to 1.3 µg/mL.

#### 3.4.7 Potency test

The test sample and Reference Hepatitis A Vaccine (hereafter referred to as “Reference” in this monograph) shall be used.

##### 3.4.7.1 Potency test on immunized animals

The test sample and the Reference shall be diluted with physiological saline to provide appropriate logarithmic serial dilutions, respectively. At least 16 mice aged 5 weeks shall be used for each dilution. 1 mL per mouse shall be given by intraperitoneal injection. The animals shall be bled at 7 weeks after immunizing injection. Anti-HAV antibody in the serum of individual animal shall be detected by enzyme immunoassay or other appropriate method.

##### 3.4.7.2 Test for antigen content in vitro

The content of HAV antigen shall be measured by enzyme immunoassay with the test sample and the Reference.

##### 3.4.7.3 Criterion for judgment

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

#### 3.4.8 Identity test

The test shall be conducted serologically.

### **4. Storage and expiry date**

Storage temperature shall be no higher than 10°C. If the solvent is frozen, the vial may be broken. The expiry date shall be three years.

### **5. Other requirements**

#### 5.1 Reconstituent

The reconstituent shall be water for injection.

#### 5.2 Information to be provided in package insert and other labeling

- (1) The reconstitution of vaccine shall be done with water for injection immediately before injection.
- (2) Recommended human dose and route of administration

This vaccine shall be dissolved in 0.65 mL of the solvent provided, and generally given in 2 doses of 0.5 mL each by subcutaneous or intramuscular injection in those aged 16 years or older with an interval of 2 to 4 weeks provided between the first and second doses and of 24 weeks between the second and third doses.

An accelerated immunization regimen shall be conducted as follows:

Two doses of 0.5 mL each shall be given by subcutaneous or intramuscular injection at an interval 2 weeks. A third dose is recommended to maintain antibody titer for an extended period.

# **FREEZE-DRIED LIVE ATTENUATED MUMPS VACCINE**

## **1. Descriptive definition**

“Freeze-dried Live Attenuated Mumps Vaccine” is a freeze-dried product containing attenuated mumps virus (hereafter referred to as “virus” in this monograph). When reconstituted, it becomes a colorless or reddish clear liquid.

## **2. Production control**

### 2.1 Source materials

#### 2.1.1 Strains of virus used for production

Strains of virus approved as suitable for production shall be used. Virus contained in the product shall be passaged under the approved culture conditions and shall not be passaged more than 5 times from the original vaccine approved as adequate for the strain for production.

#### 2.1.2 Embryonated hen eggs used for production

Chick embryos used for virus cultivation shall be harvested from chicken eggs derived from healthy flocks free from infectious diseases.

#### 2.1.3 Culture media

The cell culture medium can be supplemented with appropriate cell growth-promoting substances, phenol red at a concentration not higher than 0.002 w/v%, and minimal amounts of antibiotics, except penicillin. If non-human serum or fractions thereof are added to the medium, serum albumin concentration in the final bulk shall be controlled to be no higher than 50 ng per dose. The culture medium used for the propagation of virus can be supplemented with phenol red at a concentration not higher than 0.002 w/v%, appropriate stabilizer and minimal amounts of antibiotics. However, no non-human serum or fractions thereof, nor penicillin shall be added.

### 2.2 Bulk material

#### 2.2.1 Cell culture

Chick embryonic cell culture obtained in one session shall be regarded as an “individual cell culture”. No cytopathic change shall be detected prior to the inoculation of virus. The individual cell culture shall be subjected to the test given in 3.1.

#### 2.2.2 Virus suspension

Chick embryonic cell culture shall be used for propagation. The virus suspension harvested from individual cell cultures shall be pooled to serve as a "single harvest". The single harvest shall be tested as directed in 3.2.1. The single harvests shall be pooled to make the virus suspension. Appropriate stabilizer may be added to the virus suspension before filtration. The virus suspension before filtration shall be tested as directed in 3.2.2

#### 2.2.3 Filtration

The virus suspension before filtration shall be centrifuged and/or filtrated to eliminate cells, and the suspension shall be combined to make the bulk material. The bulk material shall be tested as directed in 3.3.

### 2.3 Final bulk and freeze-drying

The bulk material shall be diluted, if required, to make the final bulk. Appropriate stabilizer may be used. However, antibiotics shall not be added. The final bulk shall be filled into final containers and freeze-dried. The final bulk shall be tested as directed in 3.4.

### **3. Control tests**

#### 3.1 Tests on individual cell cultures

##### 3.1.1 Tests on cell cultures derived from chick embryos

A volume equivalent to 25% or 500 mL of individual cell cultures shall be used as the control cell culture of uninfected cells. The control cell culture shall be tested as given below.

##### 3.1.1.1 Observation of cell culture

The control cell cultures shall be cultivated without being inoculated with the virus under the same conditions used for virus infected cell cultures. No cytopathic change due to extraneous virus(s) shall be detected. During the observation period, control cell cultures discarded due to any nonspecific or incidental reason shall be less than 20% of the total control cells cultures.

##### 3.1.1.2 Tests in cell cultures

At the end of the above observation period, the culture medium shall be harvested from the vessel, pooled if necessary, and be tested as directed in 3.3.3.2

#### 3.2 Tests on virus suspension

##### 3.2.1 Tests on single harvest

##### 3.2.1.1 Sterility test

Sterility Test and Mycoplasma Test given in General Tests shall apply.

##### 3.2.1.2 Tests for freedom from extraneous viruses

The tests given in 3.3.3.2 shall apply. In this case, the tests shall be performed, if necessary, after neutralization of mumps virus with antiserum. The antiserum used for this purpose shall be derived from animals not of human, simian or avian origin.

##### 3.2.2 Tests on virus suspension before filtration

##### 3.2.2.1 Sterility test

Sterility Test, Mycoplasma Test, Mycobacterial Sterility Test given in General Tests shall apply. For the Mycobacterial Sterility Test, a 25-mL portion of the test sample shall be centrifuged and resuspended in 5 mL of physiological saline prior to use.

#### 3.3 Tests on bulk material

The bulk material diluted to the same concentration as the final bulk shall be tested according to the procedures given below.

##### 3.3.1 Staining test

The test given in General Tests shall apply.

##### 3.3.2 Sterility test

Sterility Test given in General Tests shall apply.

##### 3.3.3 Test for freedom from extraneous viruses

The test shall be performed after neutralization of mumps virus, if necessary, according to the

procedures given in 3.2.1.2.

### 3.3.3.1 Tests in animals

#### 3.3.3.1.1 Inoculation of adult mice

At least 10 mice aged 4–5 weeks shall be inoculated intraperitoneally with 0.5 mL and intracerebrally with 0.03 mL of the sample. The mice shall be observed for 21 days. No animal shall show evidence of infection with extraneous microbial agents, and more than 80% of the inoculated mice must survive the observation period.

#### 3.3.3.1.2 Inoculation of suckling mice

Within 24 hours after birth, suckling mice shall be inoculated intraperitoneally with 0.1 mL and intracerebrally with 0.01 mL of the test sample. The mice shall be observed for 14 days. Except those died within the first 24 hours of the test, no mouse of at least 20 mice shall show evidence of infection with extraneous microbial agents, and more than 80% of the mice must survive the observation period.

#### 3.3.3.1.3 Intracerebral inoculation of guinea pigs

At least 5 guinea pigs of 300–400 g weight shall be inoculated intracerebrally with 0.1 mL of the test sample. The guinea pigs shall be observed for 14 days. No guinea pig shall show evidence of infection with extraneous microbial agents, and more than 80% of the guinea pigs must survive the observation period.

### 3.3.3.2 Tests in cell cultures

#### 3.3.3.2.1 Inoculation of human tissue culture cells

A 10-mL portion of the test sample shall be inoculated into human cells. The inoculated cells shall be cultured for 7 days, and portions of the cells shall be passaged by culture for a further 7 days. No cytopathic change due to extraneous viruses shall be detected within the entire culture period.

#### 3.3.3.2.2 Inoculation of primary tissue cultures of embryonic chick cells

A 25-mL portion of the test sample shall be inoculated into primary cultures of chick embryo cells. The inoculated cells shall be cultured and passaged 3 times. No avian leukemia viruses shall be detected in the cultured cells or fluid using an approved method such as enzyme-linked immunosolvent assay. In addition, no reticuloendotheliosis virus antigen shall be stained immunologically in the cultured cells using the anti-reticuloendotheliosis virus serum.

#### 3.3.3.2.3 Inoculation of primary renal tissue cultures of chick cells

A 5-mL portion of the test sample shall be inoculated into primary cultures of chick kidney cells. Inoculated cells shall be incubated and observed for 14 days. Then cells shall be frozen and thawed to induce passage, and subsequently inoculated into fresh primary cultures of chick kidney cells. After observation for 14 days, the inoculated cells shall be examined by the cell-hemadsorption assay with red blood cells of guinea pigs and chickens. No cell-hemadsorption shall be detected. No cytopathic change due to extraneous viruses shall be detected during any observation period.

### 3.3.3.3 Inoculation of embryonated chicken eggs

At least 20 embryonated chicken eggs aged 10–11 days each inoculated with 0.25 mL of test sample onto the chorioallantoic membranes or into the allantoic cavity shall be incubated for 3 days. At least 20 embryonated chicken eggs aged 6–7 days each inoculated with 0.25 mL of the test sample into the yolk sac shall be incubated for 12 days. No egg, except those died within the first 24

hours of the test, shall show evidence of infection with extraneous viruses, and more than 80% of the eggs must survive the observation period. Samples obtained from eggs that died after the first 24 hours of the test shall be reinoculated into at least 10 additional eggs by the same inoculation route, and these should be incubated for the same number of days. No egg shall show evidence of infection with extraneous viruses, and more than 80% of the eggs must survive the observation period.

#### 3.3.4 Identification test

The propagation of virus in the sample shall be suppressed in appropriate cell cultures after neutralization with anti-mumps virus serum.

#### 3.3.5 Neurovirulence safety test

The test shall be performed in *Macacus* or *Cercopithecus* monkeys that have been shown to be serologically negative for mumps. At least 10 monkeys shall be employed in each test. The test sample shall be injected into the thalamic region of each hemisphere at a dose of 0.5 mL and into the cerebellomedullary cistern at a dose of 0.25 mL. The animals shall be observed for 21 days. No signs of paralysis or other neurological disorders shall be detected, and more than 80% of the animals must survive the observation period. No animal shall show abnormal clinical signs or death due to the virus or extraneous microbial agents. At the end of the observation period, each animal shall be autopsied for histopathological examination of the central nervous system. No evidence of changes due to the presence of unusual neurotropism by the virus or to the presence of extraneous microbial agents in test samples of central nervous system shall be demonstrated. In addition, animals that demonstrate the possibility of immunodeficiency as suggested by clinical signs and symptoms and histopathological findings, and those that show an underlying immunologic disease shall be excluded from the test. At least 80% of the inoculated monkeys shall be serologically positive for mumps at the end of the observation period. In consecutive tests of preparations from the same strain of the virus, no apparent differences between tests shall be demonstrated in any data from all of the animals used, including differences in the character, severity, and spread of lesions, and clinical signs during observation.

If each of the first 5 consecutive lots of bulk material prepared from a seed virus strain satisfies the requirements above, subsequent lots prepared from the same seed virus strain shall not be required to undergo the neurovirulence safety test.

#### 3.3.6 Test for virus content

The test given in 3.5.3 shall apply.

#### 3.3.7 Marker test

The plaque size of the test sample shall be the same as that of the approved strain for vaccine production used as the Reference Virus in the test.

### 3.4 Tests on final bulk

#### 3.4.1 Staining test

The test given in General Tests shall apply.

#### 3.4.2 Sterility test

The test given in General Tests shall apply.

#### 3.4.3 Test for virus content

The final bulk shall be tested for mumps virus concentration according to the procedure given

in 3.5.3

#### 3.4.4 Test for freedom from abnormal toxicity

The final bulk shall be tested for the absence of abnormal toxicity according to the procedure given in General Tests.

#### 3.5 Tests on final product

Following tests shall apply to each final lot.

##### 3.5.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the procedures given in General Tests.

##### 3.5.2 Sterility test

The test given in General Tests shall apply.

##### 3.5.3 Potency test

The concentration of mumps virus in the final product shall be determined by titration in a suitable cell culture system. The potency of the test sample shall be shown as PFU, FFU, or CCID<sub>50</sub>. The minimum acceptable virus titer per 0.5 mL shall be 5,000.

##### 3.5.4 Identity test

Mumps virus in the final product shall be identified by immunofluorescence after propagation of virus in an appropriate cell culture.

#### **4. Storage and expiry date**

The final product shall be kept at 5 °C or less during storage. The expiry date shall be one year.

#### **5. Other requirements**

##### 5.1 Reconstituent

The reconstituent shall be water for injection.

##### 5.2 Information to be provided in package insert and other labeling

- (1) Name of the virus strain used for production.
- (2) Name of the cell culture used for the cultivation of virus.
- (3) Names and concentrations of antibiotics or dyes used in the cultivation of virus, if applicable.
- (4) Names and concentrations of stabilizer, if used.
- (5) The recommended human dosage and route of administration is as follows:

Usually, 0.5 mL is given by subcutaneous injection.

# GAS GANGRENE ANTITOXIN, EQUINE

## 1. Descriptive definition

“Gas Gangrene Antitoxin” is a colorless or slightly yellowish brown clear or slightly whitish turbid liquid product containing "Clostridium perfringens (C. welchii) type A antitoxin," "Clostridium septicum (Vibrion septique) antitoxin" and "Clostridium oedematiens (C. novyi) antitoxin" in immunoglobulin of horses (hereafter referred to as the respective "antitoxin" in this monograph).

The product may also contain "Clostridium histolyticum antitoxin."

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Antigens used for immunization

Toxins or toxoids corresponding to each antitoxin shall be used for immunization.

#### 2.1.2 Animals used for production

Horses shall be used for production.

### 2.2 Bulk material

#### 2.2.1 Crude antitoxic materials

Crude antitoxic serum or plasma shall be used if it contains no less than 250 units of antitoxin per mL and passes the sterility test and the pyrogen test given in General Tests.

#### 2.2.2 Purification

Fractions containing immunoglobulin shall be prepared by fractionating the crude antitoxic material using a suitable method that has been shown not to cause the deterioration of antibodies. The fractions shall be treated with a suitable proteolytic enzyme. The preparation containing the treated antitoxin shall serve as the bulk material.

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

### 2.3 Final bulk

The bulk material shall be diluted with buffered physiological saline or other suitable medium and mixed such that 1mL of the final bulk contains no fewer than 500 units of each antitoxin.

Appropriate preservatives may be added.

## 3. Control tests

### 3.1 Tests on bulk material

#### 3.1.1 Test for immunoglobulin content

When the Cellulose Acetate Membrane Electrophoretic Test given in General Tests is applied, no less than 95% of the total proteins shall be immunoglobulin.

#### 3.1.2 Test for residual proteolytic enzyme

When measured by a suitable method for the detection of proteolytic enzyme activity, the test material shall be practically free from residual proteolytic enzyme activity.

#### 3.1.3 Sterility test

The sterility test given in General Tests shall apply.

### 3.1.4 Pyrogen test

The test given in General Tests shall apply.

### 3.1.5 Test for antitoxin content

The method given in 3.2.7 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 7.4.

### 3.2.2 Test for protein content

When the test for protein nitrogen content given in General Tests is applied, the total protein content shall be no higher than 170 mg/mL, and no higher than 150 mg per 500 units with regard to the lowest antitoxin titer among the three antitoxins.

### 3.2.3 Test for preservative content

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

### 3.2.4 Sterility test

The sterility test given in General Tests shall apply.

### 3.2.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.2.6 Pyrogen test

The test given in General Tests shall apply.

### 3.2.7 Potency tests

#### 3.2.7.1 Materials

Potency tests shall be conducted using Standard Antitoxins (hereafter referred to as "Standard" in this monograph) and test toxins corresponding to each antitoxin. Dilution of these materials shall be made with 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

#### 3.2.7.2 Test procedures

| Antitoxin  | <i>C. perfringens</i> | <i>C. septicum</i> | <i>C. oedematiens</i> | <i>C. hislolyticum</i> |
|--|-----------------------|--------------------|-----------------------|------------------------|
| A. Units per dose to be contained in median dilution | 0.2                   | 0.5                | 0.02                  | 1.0                    |
| B. Injection dose in mL                              | 0.5                   | 0.5                | 0.2                   | 0.5                    |
| C. Route of injection                                | iv                    | iv                 | im                    | iv                     |

The potency test shall be conducted with respect to each antitoxin.

Each Standard shall be diluted such that the dilutions in half of the dose indicated in row B contain five levels of antitoxin content at appropriate intervals determined with careful consideration to the accuracy of the test, the content in the median dilution being indicated in row A (hereafter referred to as "standard dilution" in this monograph).

A series of five dilutions shall be similarly made with each test sample (hereafter referred to as

"test dilution" in this monograph).

Each test toxin shall be diluted such that half of the dose indicated in row B contains one test dose (hereafter referred to as "toxin dilution" in this monograph).

A volume shall be taken accurately from each of the standard and test dilutions, mixed with an equal volume of the corresponding toxin dilution and mixed well. Each mixture shall be kept standing for 1 hour and injected into at least 3 mice aged 23–29 days by the route indicated in row C at the dose indicated in row B of the table. The animals shall be observed for 4 days after injection.

#### 3.2.7.3 Criterion for judgment

Respective antitoxin contents of the test sample shall be determined by statistical analysis of test results. The final product shall contain each antitoxin at no less than the value stated on the label.

#### 3.2.8 Identity test

The test shall be conducted by methods appropriate to the identification of the respective antitoxins.

### **4. Storage and expiry date**

The expiry date shall be three years.

### **5. Other requirements**

#### 5.1 Antitoxin contents of final containers

The sealed final container shall contain no less than 5,000 units of each antitoxin.

#### 5.2 Description on the label

Antitoxin content each antitoxin in units per mL

## **FREEZE-DRIED GAS GANGRENE ANTITOXIN, EQUINE**

### **1. Descriptive definition**

“Freeze-dried Gas Gangrene Antitoxin” is a freeze-dried product containing "*Clostridium perfringens* (*C. welchii*) type A antitoxin," "*Clostridium septicum* (*Vibrion septique*) antitoxin" and "*Clostridium oedematiens* (*C. novyi*) antitoxin" in immunoglobulin of horses (hereafter referred to as the respective "antitoxin" in this monograph).

When reconstituted, it becomes a colorless or slightly yellowish brown clear or slightly whitish turbid liquid.

The product may also contain "*Clostridium histolyticum* antitoxin."

### **2. Production control**

#### 2.1 Source materials

Requirements given in 2.1 of Gas Gangrene Antitoxin, Equine shall apply.

#### 2.2 Bulk material

Requirements given in 2.2 of Gas Gangrene Antitoxin, Equine shall apply.

#### 2.3 Final bulk and freeze-drying

The bulk material shall be pooled, diluted if necessary, with buffered physiological saline or other suitable medium to contain no less than 500 units per mL of each antitoxin, dispensed and freeze-dried.

### **3. Control tests**

#### 3.1 Tests on bulk material

The tests given in 3.1 of Gas Gangrene Antitoxin, Equine shall apply.

#### 3.2 Tests on final product

Following tests shall apply to each final lot.

##### 3.2.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

##### 3.2.2 Test for pH

The test given in 3.2.1 of Gas Gangrene Antitoxin, Equine shall apply.

##### 3.2.3 Test for protein content

When the test for protein nitrogen content given in General Tests is applied, the total protein content shall be no higher than 85 mg/mL, and no higher than 150 mg/500 units with regard to the lowest antitoxin titer among the three antitoxins.

##### 3.2.4 Sterility test

The test given in General Tests shall apply.

##### 3.2.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

##### 3.2.6 Pyrogen test

The test given in General Tests shall apply.

##### 3.2.7 Potency test

The test given in 3.2.7 of Gas Gangrene Antitoxin, Equine shall apply.

3.2.8 Identity test

The test given in 3.2.8 of Gas Gangrene Antitoxin, Equine shall apply.

**4. Storage and expiry date**

The expiry date shall be 10 years.

**5. Other requirements**

5.1 Antitoxin contents of final containers

The sealed final container shall contain no less than 5,000 units of each antitoxin.

5.2 Description on the label

Antitoxin content of each antitoxin in units per mL

# INACTIVATED RABIES VACCINE

## 1. Descriptive definition

“Inactivated Rabies Vaccine” is a whitish turbid liquid product containing inactivated rabies virus (hereafter referred to as "virus" in this monograph).

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Strain of virus used for production

The Nishigahara strain or other strain with comparative antigenicity shall be used.

#### 2.1.2 Animals used for production

Suckling mice aged 4 or younger or other appropriate animals shall be used.

### 2.2 Bulk material

#### 2.2.1 Virus suspension

When inoculated mice show complete paralysis following typical signs of infection with fixed rabies virus, their brains shall be harvested. Alternatively, virus-containing tissues shall be harvested when the virus is considered to have propagated to an adequate level.

The brain or tissue containing virus shall be mixed with buffered physiological saline or other suitable medium and thoroughly triturated. It shall be filtered or centrifuged to remove coarse particles, and then made into a 40 w/v% emulsion to serve as the virus suspension.

The virus suspension shall be subjected to the tests given in 3.1.

#### 2.2.2 Inactivation and purification

Inactivation of virus shall be conducted by the addition of an inactivator such as phenol, formalin or other suitable agent to the virus suspension, or by other suitable procedure.

After the completion of inactivation, the virus suspension shall be centrifuged at approximately  $2,000 \times g$  for 30 minutes. The supernatant shall serve as the bulk material.

The inactivation process may be conducted after centrifugation.

The bulk material shall be subjected to the tests given in 3.2.

### 2.3 Final bulk

The bulk material shall be diluted in buffered physiological saline or other suitable medium. When brain is used as the bulk material, its final concentration in the final bulk shall be no higher than 5 w/v%.

Appropriate stabilizer may be added.

The final bulk shall be subjected to the tests given in 3.3.

## 3. Control tests

### 3.1 Tests on virus suspension

#### 3.1.1 Sterility test

Sterility test and Mycoplasma test given in General Tests shall apply.

#### 3.1.2 Test for virus content

The test sample shall be diluted four-fold in 0.013 mol/L phosphate-buffered sodium chloride solution (pH 7.0). The dilution shall be further diluted 10,000-, 100,000- and 1,000,000-fold.

Each dilution shall be given by intracerebral injection at a dose of 0.03 mL into at least 10 mice aged 4 weeks. The inoculated animals shall be observed for 14 days.

The dilution factor corresponding to 1 LD<sub>50</sub> shall be no less than 100,000 upon statistical analysis.

### 3.2 Tests on bulk material

#### 3.2.1 Staining test

The test given in General Tests shall apply.

#### 3.2.2 Sterility test

The test given in General Tests shall apply.

#### 3.2.3 Inactivation test

The test given in 3.4.6 shall apply.

### 3.3 Tests on final bulk

#### 3.3.1 Test for thimerosal content

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

#### 3.3.2 Sterility test

The test given in General Tests shall apply.

#### 3.3.3 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.4 Tests on final product

Following tests shall apply to each final lot.

#### 3.4.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

#### 3.4.2 Test for thimerosal content

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

#### 3.4.3 Test for content of inactivating agent

If phenol is used for the inactivation of virus, the test given in General Tests shall apply. Phenol content shall be no higher than 0.25 w/v%.

If formalin is used for the inactivation of virus, the test given in General Tests shall apply. Formaldehyde content shall be no higher than 0.01w/v%.

#### 3.4.4 Sterility test

The test given in General Tests shall apply.

#### 3.4.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.4.6 Inactivation test

The tests shall be conducted in mice and in rabbits.

##### 3.4.6.1 Test in mice

The test sample shall be given by intracerebral injection at a dose of 0.03 mL into at least 10 mice aged 3 weeks. The inoculated animals shall be observed for 21 days. No animal shall show signs of infection with fixed rabies virus or other abnormal symptoms.

#### 3.4.6.2 Test in rabbits

The test sample shall be given by intracerebral injection at a dose of 0.25 mL into at least 2 rabbits weighing 1.5–2.5 kg. The inoculated animals shall be observed for 21 days. No animal shall show signs of infection with fixed rabies virus or other abnormal symptoms.

#### 3.4.7 Potency test

The test shall be conducted by a method with varying challenge doses or a method with varying immunizing doses, as follows.

##### 3.4.7.1 Test with varying challenge doses

###### 3.4.7.1.1 Materials

The test sample and CVS strain of virus for challenge shall be used.

The test sample shall be diluted 10-fold in 0.013 mol/L phosphate-buffered sodium chloride solution (pH 7.0). The brains of mice injected with the CVS strain shall be made generally into a 20 w/v% emulsion in physiological saline containing 2 w/v% horse serum to serve as stock suspension of virus for challenge. The stock suspension of virus for challenge shall be stored frozen at  $-40^{\circ}\text{C}$  or less.

The frozen stock suspension of virus for challenge shall be thawed, diluted two-fold with physiological saline containing 2 w/v% horse serum, and centrifuged at about  $2,000 \times g$  for 10 minutes.

The supernatant shall be diluted 1,000-fold. The dilution shall be given by intracerebral injection into mice at a dose of 0.03 mL. The brains of mice showing typical signs of infection with fixed rabies virus for at least 24 hours shall be harvested and made into a 20 w/v% emulsion in physiological saline containing 2 w/v% horse serum (the emulsion shall be stored frozen at  $-40^{\circ}\text{C}$  or less).

The emulsion can be diluted two-fold and centrifuged. The supernatant shall be used as 10-fold dilution and then be diluted  $10^1$ -,  $10^2$ -,  $10^3$ -,  $10^4$ - and  $10^5$ -fold to serve as virus suspensions for challenge.

###### 3.4.7.1.2 Test procedures

The test sample shall be given by intraperitoneal injection at 6 doses of 0.25 mL into 5 groups of at least 10 mice aged 4 weeks each every second day. Fourteen days after the first immunizing injection, each group shall be given by intracerebral injection with the diluted sample at a volume dose of 0.03 mL. The challenged animals shall be observed for 14 days.

At least three serial dilutions of the virus suspensions for challenge shall be given by intracerebral injection at a dose of 0.03 mL into other groups of at least 10 non-immune mice. The inoculated animals shall be observed for 14 days. The dilution factor of the challenge suspension required to obtain 1  $\text{LD}_{50}$  is measured.

Any animal showing paralysis on the last day of the observation period shall be included in deaths.

###### 3.4.7.1.3 Criterion for judgment

The dilution factor of the challenge suspension required to obtain 1  $\text{LD}_{50}$  per 0.03 mL for the

immunized animals shall be no greater than 1/1,000 of that for non-immune animals upon statistical analysis.

#### 3.4.7.2 Test with varying immunizing doses

##### 3.4.7.2.1 Materials

The test sample, Reference Inactivated Rabies Vaccine (hereafter referred to as "Reference" in this monograph) and CVS strain of virus for challenge shall be used.

The diluent for the test sample and the Reference shall be 0.013 mol/L phosphate-buffered sodium chloride solution (pH 7.0).

The methods provided in 3.4.7.1.1 shall apply to the preparation and storage of the virus suspension for challenge.

The stored suspension shall be thawed and diluted in physiological saline containing 2 w/v% horse serum such that the virus suspension for challenge containing viruses having about 25 LD<sub>50</sub> per 0.03 mL is prepared.

##### 3.4.7.2.2 Test procedures

The test sample and the Reference shall each be diluted five-fold serially into four levels.

Each dilution shall be given by intraperitoneal injection into at least 10 mice aged 4 weeks in 2 doses of 0.5 mL at a weekly interval.

Two weeks after the first immunizing injection, each animal shall be given by intracerebral injection with 0.03 mL of the challenge virus suspension. The animals shall be observed for 14 days.

Appropriate dilutions of the challenge virus suspension shall be given by intracerebral injection at a dose of 0.03 mL into at least 10 non-immune mice. The animals shall be observed for 14 days. Any animal showing paralysis on the last day of the observation period shall be included in deaths.

The challenge virus suspension shall contain 10–100 LD<sub>50</sub> per 0.03 mL.

##### 3.4.7.2.3 Criterion for judgment

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

#### 3.4.8 Identity test

The supernatant of the test sample obtained by high-speed centrifugation shall be tested by a serological technique using rabies immune serum.

### **4. Storage and expiry date**

The expiry date shall be six months.

### **5. Other requirements**

#### 5.1 Containers of the final product

Usually, 2 mL ampoules shall be used.

#### 5.2 Information to be provided in package insert and other labeling

- (1) Species of animal used for production
- (2) Method for the inactivation of virus

(3) Recommended human dose and route of administration, as follows:

- 1) Preparatory injections (to be conducted at the time of injury by an animal suspected to be infected with rabies but not yet confirmed by laboratory diagnosis. When the animal is found not to be infected with rabies, the injections shall immediately be discontinued. When the animal is found to be infected with rabies, the complete injections shall be conducted)

A dose of 0.2 mL of the vaccine preparation shall be given by intracutaneous injection once daily for 7 days.

- 2) Complete injections (to be conducted if the person is suspected to be infected with rabies)

A dose of 0.2 mL of the vaccine preparation shall be given by subcutaneous (not intramuscular) injection once daily for 14 days.

- 3) Emergency injections (to be conducted if bitten on the neck, head, or face, or heavily injured in other part of the body when infection with rabies is suspected)

Two doses of 2 mL each injected subcutaneously once daily for 7 days, followed by complete injections given in (2).

4) Others

- (a) Children receive the same dose as adults.
- (b) When injection to be conducted in those who had received rabies vaccination before, the proper method shall apply as described in (1), (2) and (3). It is necessary to pay attention to possible side effects from the time of the first vaccination. Those who have received the vaccination within the preceding 6 months are not required to receive it again at the time of repeated vaccination.
- (c) When injection is to be conducted in those aged over 10 years, the possibility exists of side effects such as post-vaccination paralysis, etc. Selection of injection methods shall be dependent on the presence of rabies outbreaks in the area, site of bite injury, level of injury, history of rabies vaccination, observation of the biting animal and laboratory diagnosis.

# **FREEZE-DRIED INACTIVATED TISSUE CULTURE RABIES VACCINE**

## **1. Descriptive definition**

“Freeze-dried Inactivated Tissue Culture Rabies Vaccine” is a freeze-dried product containing inactivated rabies virus (hereafter referred to as "virus" in this monograph). When reconstituted, it becomes a colorless or slightly yellowish-red clear liquid.

## **2. Production control**

### **2.1 Source materials**

#### **2.1.1 Strains of virus used for production**

Strains of virus approved for production shall be used.

#### **2.1.2 Eggs**

Chick embryos used for virus cultivation shall be harvested from eggs of healthy flocks free from infectious disease.

#### **2.1.3 Culture media**

The growth medium suitable for culturing chick embryo cells shall be used. The growth medium can be supplemented with adequate growth promoting substances, phenol red at a concentration of not higher than 0.002 w/v%, and minimal amounts of antibiotics; however, penicillin shall not be added. If heterologous serum or its fraction is supplemented for growth promotion, an appropriate treatment is necessary to adjust the serum albumin content in the final bulk to not higher than 50 ng per dose of vaccine.

The medium suitable for the preparation of each strain of virus shall be used. The medium for virus propagation can be supplemented with phenol red at a concentration not higher than 0.002 w/v%, appropriate stabilizer, and minimal amounts of antibiotics. No heterologous serum nor its fraction or penicillin shall be added.

### **2.2 Bulk material**

#### **2.2.1 Cell culture**

Chick embryo cell cultures handled in one session shall be regarded as a single cell culture. No cytopathic change shall be detected prior to the inoculation of virus. The individual cell cultures shall be subjected to the tests given in 3.1.

#### **2.2.2 Virus suspension**

The pooled virus suspensions harvested from individual cell cultures, in which cultured cells were separated by centrifugation, filtration or other appropriate treatment, shall serve as a single virus suspension.

The single virus suspension shall be subjected to the tests given in 3.2.1.

#### **2.2.3 Inactivation and purification**

The single virus suspension inactivated by an appropriate method is regarded as an inactivated virus suspension. The inactivated virus suspension shall be subjected to the tests given in 3.2.2.

The inactivated virus suspensions shall be pooled, purified, concentrated if necessary, and regarded as the bulk material.

The bulk material shall be subjected to the tests given in 3.3.

## 2.3 Final bulk and freeze-drying

The bulk material shall be mixed and, if necessary, diluted to serve as the final bulk.

Appropriate stabilizer may be added.

The final bulk shall be dispensed into final containers and freeze-dried.

## 3. Control tests

### 3.1 Tests on cell cultures

#### 3.1.1 Tests on chick embryo-cell cultures

A suitable quantity equivalent to 5% or 500 mL of individual cell cultures shall be used as the control cell culture. The control cell culture shall be subjected to the tests as given below.

##### 3.1.1.1 Observation of cell culture

The control cell culture shall be incubated without being inoculated with virus under the conditions same for virus-infected cell culture. No cytopathic change due to extraneous virus shall be detected. During the observation period, cultures discarded due to non-specific or incidental reason shall be less than 20% of the total control cell cultures.

##### 3.1.1.2 Inoculation test in chick primary embryo cell cultures

At the end of the above observation period, the medium shall be harvested from each vessel, pooled if necessary, and 25 mL of the pooled cultured medium shall be inoculated to the chick embryo cell culture. After 3-time passages, no avian leukemia virus shall be detected by enzyme-linked immunosorbent assay or other appropriate method.

When cells are tested by immunofluorescence test using anti-reticuloendotheliosis virus immune serum, no reticuloendotheliosis virus antigens shall be detected in the 3-time passaged cells.

### 3.2 Tests on virus suspension

#### 3.2.1 Tests on single harvest

##### 3.2.1.1 Sterility test

Sterility test and Mycoplasma test given in General Tests shall apply.

##### 3.2.2 Test on inactivated virus suspension

##### 3.2.2.1 Sterility test

The test given in General Tests shall apply.

##### 3.2.2.2 Inactivation test

The test given in 3.4.6 shall apply.

### 3.3 Tests on bulk material

#### 3.3.1 Staining test

The test given in General Tests shall apply.

#### 3.3.2 Sterility test

The test given in General Tests shall apply.

### 3.4 Tests on final product

Following tests shall apply to each final lot.

#### 3.4.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### 3.4.2 Test for pH

The pH shall be within the range of 6.8 to 7.4 when determined following the procedure given in General Tests.

#### 3.4.3 Test for protein nitrogen content

The protein nitrogen content shall be no higher than 40 µg/mL when determined following the procedure given in General Tests.

#### 3.4.4 Sterility test

The test given in General Tests shall apply.

#### 3.4.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.4.6 Inactivation test

The test sample shall be given by intracerebral injection at a dose of 0.02 mL each into at least 30 suckling mice aged 4 days or younger. The inoculated animals shall be observed for 21 days. No animal shall show a sign or death of fixed rabies infection.

#### 3.4.7 Potency test

The tests shall be conducted by the method at varying doses of immunization.

##### 3.4.7.1 Materials

The test sample, Reference Inactivated Rabies Vaccine (hereafter referred to as "Reference" in this monograph) and CVS strain of virus for challenge shall be used.

The diluent for the test sample and the Reference shall be an appropriate phosphate buffered sodium chloride solution.

The brains of the mice injected with the CVS strain shall be prepared, as a rule, as a 10 or 20 w/v% emulsion in phosphate buffered sodium chloride solution containing 2 vol% fetal bovine serum to serve as a stock suspension of virus for challenge. This stock virus suspension for challenge shall be stored frozen at -70°C or less. The stored suspension shall be thawed and diluted in physiological saline containing 2 vol% fetal bovine serum, and, if necessary, dilute the supernatant of the centrifuge of the emulsified brain from animals infected with CVS strain to provide a virus suspension for challenge containing approximately 25 LD<sub>50</sub> of virus per 0.03 mL.

##### 3.4.7.2. Test procedures

The test sample and the Reference shall be diluted serially five-fold in four levels at each dilution.

Each dilution solution shall be given by intraperitoneal injection into at least 10 mice aged 4 weeks at 0.5 mL twice at a weekly interval. Two weeks after the first immunizing injection, each animal shall be given by intracerebral injection with 0.03 mL of the challenge virus suspension. The animals shall be observed for 14 days.

Separately, the challenge virus suspension appropriately diluted serially shall be given by intracerebral injection at a dose of 0.03 mL into at least 10 non-immune mice. The animals shall be observed for 14 days.

Animals showing paralysis by the last day of observation shall be included in the group of deaths.

The challenge virus suspension shall contain 10–200 LD<sub>50</sub> of virus in each 0.03 mL.

#### 3.4.7.3 Criterion for judgment

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

#### 3.4.8 Identity test

The test shall be performed by a serological method.

### 4. Storage and expiry date

The expiry date shall be three years.

### 5. Other requirements

#### 5.1 Information to be provided in package insert and other labeling

- (1) Reconstitution of the vaccine immediately before use using water for injection
- (2) Names and contents of inactivating agents
- (3) Names and contents of antibiotics or dyes used in the cultivation of the virus, if applicable.
- (4) Recommended human dose and route of administration, as follows:

- 1) Pre-exposure immunization

A dose of 1 mL of the vaccine preparation shall be given by subcutaneous injection twice at a 4-week interval and once after 6–12 months as a booster.

- 2) Post-exposure treatment

A dose of 1 mL of the vaccine preparation shall be given by subcutaneous injection 6 times on Days 0, 3, 7, 14, 30 and 90.

- (a) Children shall receive the same dose as adults.
- (b) Adults or children who have received post-exposure vaccination within the preceding 6 months are required to receive it again for bite injury. If more than 6 months have elapsed since pre-exposure, those who have had bite injury are recommended to receive the revaccination in the same manner as the first vaccination.

# CHOLERA VACCINE

## 1. Descriptive definition

“Cholera Vaccine” is a whitish turbid liquid product containing inactivated *Vibrio cholerae* of strains of the Ogawa and Inaba serotypes (hereafter referred to as the respective "vibrio" in this monograph).

Monotypic products may be manufactured, if necessary.

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Strains of vibrio used for production

S phase strains of Ogawa and Inaba serotypes shall be used.

#### 2.1.2 Culture medium used for production

The medium used for cultivation of vibrio shall not contain any ingredient possibly inducing pronounced allergic reaction in humans.

### 2.2 Bulk material

#### 2.2.1 Suspension of vibrio

The vibrios shall be cultured at  $36 \pm 1^{\circ}\text{C}$  for a period not longer than 24 hours.

The cultured vibrios shall be harvested and suspended in buffered physiological saline or other suitable medium. The suspension shall be tested microscopically or by appropriate culture methods. If no contamination or any growth of R type colonies is detected, it shall serve as the vibrio suspension.

#### 2.2.2 Inactivation

Inactivation of vibrios shall be conducted by the addition of formalin, phenol, or thimerosal, by heating, or any other suitable procedure.

If formalin is used for inactivation, residual formaldehyde shall be neutralized or removed using an appropriate method. A suspension containing inactivated vibrios of a strain shall serve as the bulk material and shall be subjected to the tests given in 3.1.

### 2.3 Final bulk

The bulk material of each strain shall be diluted in buffered physiological saline or other suitable medium so that it contains  $4 \times 10^9$  vibrios of each serotype per mL when determined by the test given in 3.1.1. Appropriate preservatives may be added.

## 3. Control tests

### 3.1 Tests on bulk material

#### 3.1.1 Test for vibrio content

The test for optical density given in General Tests shall apply to the test sample within 6 hours after harvest and before the initiation of the inactivation procedure. The optical density of a test sample containing  $4 \times 10^9$  of vibrios per mL shall correspond to 10 opacity units.

### 3.1.2 Staining test

The test given in General Tests shall apply.

### 3.1.3 Test for vibrio stability in suspension

The test sample shall show no signs of aggregation of vibrios when kept standing at about 37°C for 5 hours.

### 3.1.4 Sterility test

The test given in General Tests 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 7.4.

### 3.2.2 Test for protein nitrogen content

When the test given in General Tests is applied, the protein nitrogen content shall be no higher than 100 µg/mL.

### 3.2.3 Test for preservative content

If thimerosal is used as a preservative, the test given in General Tests shall apply.

If phenol is used, the test given in General Tests shall apply.

### 3.2.4 Test for formaldehyde content

When formalin is used for the inactivation and detoxification of vibrios, the test sample shall be subjected to the test given in General Tests. Formaldehyde content shall be no more than 0.01 w/v%.

### 3.2.5 Sterility test

The test given in General Tests shall apply.

### 3.2.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.2.7 Test for toxicity to mouse weight gain

The test sample shall be given by intraperitoneal injection into at least 5 mice aged 5 weeks at a dose of 0.5 mL per mouse. The animals shall be observed for 7 days. The mean body weight 3 days after injection shall be no less than that at the time of injection upon statistical analysis. No animal shall show any abnormal signs during the observation period.

### 3.2.8 Potency test

Potency shall be determined in mice by the intraperitoneal challenge method with live vibrios of suitable strains suspended in a mucin solution.

#### 3.2.8.1 Materials

The test sample, Reference Cholera Vaccine (Ogawa serotype), Reference Cholera Vaccine (Inaba serotype) (hereafter referred to as the respective "Reference" in this monograph) and a suitable challenge strain of each serotype shall be used. The diluent for the test sample and the Reference shall be 0.013 M phosphate-buffered sodium chloride solution.

Each of the challenge strains shall be cultured at  $36 \pm 1^\circ\text{C}$  for about 5 to 18 hours, and made into a suspension of a 5 to 10 w/v% mucin solution containing live vibrios of approximately 1,000

LD<sub>50</sub> per 0.5 mL when injected intraperitoneally into mice. A vibrio suspension of each serotype shall serve as challenge suspension.

#### 3.2.8.2 Test procedures

The test sample and Reference shall be diluted to make three levels of 10-fold or other appropriate logarithmic serial dilutions. Each dilution of the test sample shall be given by intraperitoneal injection at a dose of 0.5 mL per mouse into 2 groups of at least 10 mice each aged 4 weeks. Each dilution of the Reference shall be injected to the animals in 1 group of at least 10 mice in the same manner. At 12–16 days after the immunizing injection, 1 of the 2 groups of mice injected with the test sample dilution shall be given by intraperitoneal injection with the vibrio suspension of the Ogawa serotype, the other with the challenge suspension of the Inaba serotype, and those immunized with the Reference dilution with the challenge suspension of the corresponding serotype, all at a dose of 0.5 mL per mouse. All the animals shall be observed for 3 days.

Separately, the vibrio suspension for challenge shall be serially diluted into 3 or more appropriate levels and each dilution shall be given by intraperitoneal injection into a group of at least 10 mice aged 6 weeks to titrate the virulence. The value of a vibrio suspension for challenge divided by 1000 shall be between the upper and lower confidence limit value of LD<sub>50</sub>.

In this test, animals of the same sex shall be used for each serotype.

#### 3.2.8.3 Criterion for judgment

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

#### 3.2.9 Identity test

Identity shall be tested based on agglutination or precipitation in tubes using immune sera of the anti-Ogawa and anti-Inaba serotypes.

### **4 Storage and expiry date**

The expiry date shall be one year.

### **5. Other requirements**

#### 5.1 Modification of the proper name

For a monotypic product, the serotype of vibrio contained in the product such as the “Ogawa serotype” shall be added to the proper name.

#### 5.2 Information to be provided in package insert and other labeling

- (1) Names of strains contained in the product
- (2) The method used for inactivation
- (3) Caution that the product be rendered homogenous by thorough shaking before use
- (4) Recommended human dose and route of administration, as follows:

Generally, a dose of 0.5 or 1.0 mL is given by twice by subcutaneous injection for adults at an interval of 5–7 days. Doses shall be 0.35 or 0.7 mL for those aged 7–13 years, 0.25 or 0.5 mL for those aged 4–7 years and 0.1 or 0.25 mL for those aged below 4 years.

# **FREEZE-DRIED DIPHTHERIA ANTITOXIN, EQUINE**

## **1. Descriptive definition**

“Freeze-dried Diphtheria Antitoxin” is a freeze-dried horse immunoglobulin containing "diphtheria antitoxin" (hereafter referred to as "antitoxin" in this monograph). When reconstituted, it becomes a colorless or slightly yellowish brown clear or slightly whitish turbid liquid.

## **2. Production control**

### 2.1 Source materials

#### 2.1.1 Antigens used for immunization

Diphtheria toxin or diphtheria toxoid shall be used for immunization.

#### 2.1.2 Animals used for production

Horses shall be used for the production of sera.

### 2.2 Bulk material

#### 2.2.1 Crude antitoxic material

Crude antitoxic serum or plasma shall be used if it contains no less than 350 units of antitoxin per mL and passes the sterility test and the pyrogen test given in General Tests.

#### 2.2.2 Purification

The fractions containing immunoglobulin shall be prepared by fractionating the crude antitoxic material using a suitable method that has been shown not to cause the deterioration of antibodies. The fraction shall be treated with a proteolytic enzyme. The preparations containing the treated antitoxin shall serve as the bulk material.

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

### 2.3 Final bulk and freeze-drying

The bulk material shall be diluted, if necessary, with buffered physiological saline or other suitable medium to contain no fewer than 500 units per mL of antitoxin, dispensed and freeze-dried.

## **3. Control tests**

### 3.1 Tests on bulk material

#### 3.1.1 Test for immunoglobulin content

When the Cellulose Acetate Membrane Electrophoretic Test given in General Tests is applied, no less than 95% of the total proteins shall be immunoglobulin.

#### 3.1.2 Test for freedom from residual proteolytic enzyme.

The test material shall be practically free from residual proteolytic enzyme activity when measured by a suitable method for the detection of proteolytic enzyme.

#### 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.

#### 3.1.5 Test for antitoxin content

The test given in 3.2.7 shall apply.

### 3.2 Tests on final product

Following tests shall apply to each final lot.

#### 3.2.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### 3.2.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

#### 3.2.3 Test for protein content

When the test for protein nitrogen content provided in General Tests is applied, the protein content shall be no higher than 30 mg per 500 units of antitoxin.

#### 3.2.4 Sterility test

The test given in General Tests shall apply.

#### 3.2.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.2.6 Pyrogen test

The test given in General Tests shall apply.

#### 3.2.7 Potency test

##### 3.2.7.1 Materials

The test sample, Standard Diphtheria Antitoxin (hereafter referred to as "Standard" in this monograph) and Diphtheria Test Toxin shall be used. Dilution of these materials shall be made with 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

##### 3.2.7.2 Test procedures

The Standard shall be diluted to five levels at appropriate intervals determined with careful consideration to the accuracy of the test, containing 1.0 unit per 2 mL for the median dilution (hereafter referred to as "standard dilution" in this monograph). The test sample shall be similarly diluted (hereafter referred to as "test dilution" in this monograph).

The test toxin shall be diluted so as to contain 1 test dose per 2 mL (hereafter referred to as "toxin dilution" in this monograph).

A volume of 2 mL shall be taken accurately from each of the standard and test dilutions, combined with 2 mL of toxin dilution and mixed well. After being allowed to stand for 1 hour, the whole content of each mixture shall be given by subcutaneous injection into guinea pigs weighing 225-275 g. At least 4 animals shall be used for each of the standard and test dilutions. The animals shall be observed for 5 days after injection.

##### 3.2.7.3 Criterion for judgment

The antitoxin content of each test sample shall be determined by statistical analysis of assay results. The final product shall contain antitoxin at no less than the value stated on the label.

#### 3.2.8 Identity test

The test shall be conducted by methods appropriate to the identification of the respective antitoxins.

### **4. Storage and expiry date**

The expiry date shall be 10 years.

### **5. Other requirements**

#### 5.1 Antitoxin content of final containers

A sealed final container shall contain an amount of antitoxin of no less than 1,500 units.

#### 5.2 Labeling

Antitoxin content in units per mL

# DIPHThERIA TOXOID

## 1. Descriptive definition

“Diphtheria Toxoid” is a colorless or slightly yellowish brown clear liquid product containing "diphtheria toxoid" (hereafter referred to as "toxoid" in this monograph), which is prepared by treating diphtheria toxin (hereafter referred to as "toxin" in this monograph) with formaldehyde (hereafter referred to as "toxoiding" in this monograph) by minimally impairing the immunogenicity of the toxin.

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Strains of *Corynebacterium diphtheriae*

*Corynebacterium diphtheriae* strain Park-Williams No. 8 or other strains with equivalent or greater toxigenicity shall be used.

#### 2.1.2 Culture medium

The medium used for the production of toxin shall be free from any substances of horse meat or human origin, specific human blood group substances, and other substances possibly inducing severe allergic reactions in human.

### 2.2 Bulk material

#### 2.2.1 Toxin filtrate

Cultures of *C. diphtheriae* after incubation shall be tested for bacterial purity by microscopic examination or by inoculation into an appropriate culture medium. The culture shown to be free from any contaminant microorganisms shall be subjected to an appropriate procedure to remove the corynebacterial cells from the culture liquid containing toxin, and this preparation shall serve as a toxin solution.

The toxin filtrate shall contain no lower than 100 Lf per mL of toxin when tested by the method given in 3.2.8.

#### 2.2.2 Toxoiding and purification

Formaldehyde shall be used for toxoiding. Purification shall be performed either before or after toxoiding. The preparation containing purified toxoid shall serve as the bulk material.

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

### 2.3 Final bulk

The bulk material shall be diluted in buffered physiological saline or other suitable medium to a concentration of not higher than 70 Lf of toxoid per mL to serve as the final bulk. Any Appropriate stabilizer may be added.

## 3. Control tests

### 3.1 Tests on bulk material

#### 3.1.1 Purity test

Each bulk material shall be tested for protein nitrogen content by the test given in General Tests and for toxoid content by the method given in 3.2.8. The bulk material shall contain no less than 1,500 Lf toxoid of per mg protein nitrogen.

#### 3.1.2 Sterility test

The test given in General Tests shall apply.

### 3.1.3 Detoxification test

The test shall be conducted on two kinds of the sample: the one shall be prepared by diluting the test sample with 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) to the concentration of 200 Lf/mL, and the other to a concentration higher than that of the final bulk not exceeding 70 Lf/mL. The latter sample shall be preserved at 37°C for 20 days prior to the test.

#### 3.1.3.1 Test in guinea pigs

The test given in 3.2.6.1 shall apply. The test sample containing 200 Lf/mL of toxoid shall be injected into animals at a dose of 2 mL.

#### 3.1.3.2 Test in rabbits

The test given in 3.2.6.2 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 6.6 to 7.4.

#### 3.2.2 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

#### 3.2.3 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%.

#### 3.2.4 Sterility test

The test given in General Tests shall apply

#### 3.2.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.2.6 Detoxification test

Following test shall be conducted on the samples with and without preservation at 37°C for 20 days.

##### 3.2.6.1 Test in guinea pigs

Each sample shall be given by subcutaneous injection at a dose of 5mL into at least 4 guinea pigs weighing 300–400 g. The animal shall be observed for at least 30 days. No animal shall die due to intoxication or show any specific symptoms of intoxication such as necrosis, paralysis, pronounced decreased body weight, or other abnormal signs during the observation period.

##### 3.2.6.2 Test in rabbits

Each sample and the Schick Test Toxin for animal use diluted 40-fold in 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing gelatin at 0.2 w/v% shall be given by intracutaneous injection at a dose of 0.1 mL into at least 2 rabbits weighing about 2.0–4.0 kg. The animals shall be observed for 2 days.

The site injected with the dilution of Schick Test Toxin shall show specific reactions to toxin, and no site injected with each sample shall show the specific or other abnormal reactions.

#### 3.2.7 Potency test

Potency shall be determined in guinea pigs either by the toxin challenge method or by the antitoxin titration method or in mice by the antitoxin titration method.

##### 3.2.7.1 Toxin challenge method

###### 3.2.7.1.1 Materials

The test sample, Standard Diphtheria Toxoid (hereafter referred to as "Standard" in this monograph) and an appropriate toxin solution shall be used. The test sample and the Standard shall be diluted with 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.02 w/v% gelatin, and the toxin solution shall be diluted with 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

#### 3.2.7.1.2 Test procedures

The test sample and the Standard shall be diluted serially at equal logarithmic intervals. Each dilution shall be given by subcutaneous injection at a dose of 2 mL into at least 10 guinea pigs weighing 400–600 g.

The animals shall be challenged with approximately 50 LD<sub>50</sub> of toxin after 4–6 weeks of immunizing injection. The animals shall be observed for 7 days.

The toxin used for challenge shall be titrated by injecting with at least three serial dilutions into at least 3 guinea pigs weighing about 400 g. The challenge toxin shall contain 25–100 LD<sub>50</sub> toxin per inoculum.

#### 3.2.7.1.3 Criterion for judgment

The potency of the test sample shall be no less than 3 IU upon statistical analysis.

#### 3.2.7.2 Antitoxin titration method

The antitoxin content of the serum shall be titrated by the rabbit intracutaneous method, cell culture method, or passive hemagglutination method.

##### 3.2.7.2.1 Materials

The test sample, the Standard, Standard Diphtheria Antitoxin and a toxin solution with a known binding capacity shall be used. When the passive hemagglutination method is used, red blood cells shall be sensitized with diphtheria toxin or toxoid of no less than 2,500 Lf per mg protein nitrogen.

##### 3.2.7.2.2 Test procedures

Animals shall be immunized by the method given in 3.2.7.1.2. Each dilution shall be given by subcutaneous injection at a dose of 0.5 mL into at least 10 mice aged 5 weeks using mouse method.

Serum content of antitoxin shall be determined after 4 to 6 weeks of immunization.

##### 3.2.7.2.3 Criterion for judgment

The criterion provided in 3.2.7.1.3 shall apply.

#### 3.2.8 Identity test

The test shall be conducted by the flocculation test.

### 4. Storage and expiry date

The expiry date shall be two years.

### 5. Other requirements

#### 5.1 Information to be provided in package insert and other labeling

Recommended human dose and route of administration, as follows:

For primary immunization, usually 3 doses of 0.5 mL are given by subcutaneous injection at 3–8 weeks intervals. To the humans aged older than 10 years, a dose of 0.1 mL shall be injected primarily and then, if untoward reactions are absent or mild, adequately increased doses shall be injected for subsequent inoculations. For the first booster immunization, usually 0.5 mL is given by subcutaneous injection with the interval of not shorter than 6 months after the completion of the primary immunization (in general, 12–18 months after the completion of the primary immunization).

To the humans showing severe untoward reactions at the primary immunization, the dose shall be adequately reduced. This precaution shall be taken for subsequent booster immunizations.

The dose for the humans aged older than 10 years shall be no more than 0.1 mL.

## ADSORBED DIPHTHERIA TOXOID

### 1. Descriptive definition

“Adsorbed Diphtheria Toxoid” is a liquid product of "diphtheria toxoid" (hereafter referred to as "toxoid" in this monograph), which is prepared by treating diphtheria toxin with formaldehyde by a method minimally impairing the immunogenicity of the toxin and rendering insoluble by the addition of aluminium salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

### 2. Production control

#### 2.1 Source materials

Requirements given in 2.1 of Diphtheria Toxoid shall apply.

#### 2.2 Bulk material

Requirements given in 2.2 of Diphtheria Toxoid shall apply.

#### 2.3 Final bulk

The bulk material shall be diluted with buffered physiological saline or other suitable medium and supplemented with aluminium salt to serve as the final bulk. The toxoid content shall be no higher than 50 Lf per mL. Any Appropriate stabilizer may be added.

### 3. Control tests

#### 3.1 Tests on bulk material

Requirements given in 3.1 of Diphtheria Toxoid 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 5.4 to 7.4.

##### 3.2.2 Test for aluminium content

When the test given in General Tests is applied, the aluminium content shall be no higher than 1.0 mg/mL.

##### 3.2.3 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

##### 3.2.4 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%.

##### 3.2.5 Sterility test

The test given in General Tests shall apply.

##### 3.2.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

##### 3.2.7 Detoxification test

The test given in 3.2.6 of Diphtheria Toxoid shall apply.

##### 3.2.8 Potency test

The test given in 3.2.7 of Diphtheria Toxoid shall apply; however, the Standard Diphtheria Toxoid in 3.2.7.1.1 shall read Standard Adsorbed Diphtheria Toxoid. The diluent for the test sample and the Standard shall be physiological saline. The potency of the test sample required in 3.2.7.1.3 of

Diphtheria Toxoid shall be no less than 70 IU/mL.

#### 3.2.9 Identity test

The test given in 3.2.8 of Diphtheria Toxoid shall apply on the test sample, which is solubilized with sodium citrate or other suitable reagent.

#### 4. Storage and expiry date

The expiry date shall be two years.

#### 5. Other requirements

##### 5.1 Information to be provided in package insert and other labeling

- (1) Caution that the product be rendered homogenous by thorough shaking before use
- (2) Recommended human dose and route of administration, as follows:

For Primary immunization, usually 2 doses of 0.5 mL are given by subcutaneous injection at a 3–8 weeks interval. To the humans aged older than 10 years, a dose of 0.1 mL shall be primarily injected and, if untoward reactions are absent or mild, an adequately increased dose shall be used for subsequent injection. For the first booster immunization, usually a dose of 0.5 mL is given by subcutaneous injection with the interval of not shorter than 6 months after the completion of the primary immunization (in general, 12–18 months after the completion of the primary immunization). To the humans showing severe reactions at the time of primary immunization, the dose shall be adequately reduced. This precaution shall be taken for subsequent booster immunizations. The dose for the humans aged older than 10 years shall be no more than 0.1 mL

## **ADSORBED DIPHTHERIA TOXOID FOR ADULT USE**

### **1. Descriptive definition**

“Adsorbed Diphtheria Toxoid for Adult Use” is a liquid product containing "diphtheria toxoid" (hereafter referred to as "toxoid" in this monograph), which is prepared by treating diphtheria toxin with formaldehyde by a method minimally impairing the immunogenicity of the toxin, and the product contains very few antigenic substances other than the toxoid. The product is rendered insoluble by the addition of aluminium salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

### **2. Production control**

#### **2.1 Source materials**

Requirements given in 2.1 of Diphtheria Toxoid shall apply.

#### **2.2 Bulk material**

Requirements given in 2.2 of Diphtheria Toxoid shall apply.

#### **2.3 Final bulk**

Requirements given in 2.3 of Adsorbed Diphtheria Toxoid shall be applied, except that the toxoid content shall be no higher than 5 Lf/mL.

### **3. Control tests**

#### **3.1 Tests on bulk material**

The tests given in 3.1 of Diphtheria Toxoid shall apply, except that the purity shall be no less than 2,500 Lf instead of 1,500 Lf per mg protein nitrogen as specified in 3.1.1.

#### **3.2 Tests on final product**

The tests given in 3.2 of Adsorbed Diphtheria Toxoid shall apply, except that the potency of the test sample in 3.2.8 shall be no less than 15 IU.

### **4. Storage and expiry date**

The expiry date shall be three years.

### **5. Other requirements**

#### **5.1 Information to be provided in package insert and other labeling**

- (1) Caution that the product be rendered homogenous by thorough shaking before use
- (2) Recommended human dose and route of administration, as follows:

Usually, the product is used to humans aged more than 10 years Injection shall be made subcutaneously at a dose of no more than 0.5 mL.

## **DIPHTHERIA-TETANUS COMBINED TOXOID**

### **1. Descriptive definition**

“Diphtheria-Tetanus Combined Toxoid” is a colorless or slightly yellowish brown clear liquid product containing "diphtheria toxoid" and "tetanus toxoid," which are prepared by treating diphtheria toxin and tetanus toxin, respectively, with formaldehyde by a method minimally impairing the immunogenicity of the toxins.

### **2. Production control**

#### 2.1 Source materials

Requirements given in 2.1 of Diphtheria Toxoid and 2.1 of Tetanus Toxoid shall apply.

#### 2.2 Bulk material

Requirements given in 2.2 of Diphtheria Toxoid and 2.2 of Tetanus Toxoid shall apply.

#### 2.3 Final bulk

The bulk material shall be diluted with buffered physiological saline or other suitable medium and mixed to make the diphtheria toxoid content not higher than 70 Lf/mL and the tetanus toxoid content not higher than 50 Lf/mL to obtain as the final bulk.

Appropriate preservatives and stabilizer may be added.

### **3. Control tests**

#### 3.1 Tests on bulk material

The tests given in 3.1 of Diphtheria Toxoid and 3.1 of Tetanus Toxoid 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 6.6 to 7.4.

##### 3.2.2 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

##### 3.2.3 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%.

##### 3.2.4 Sterility test

The test given in General Tests shall apply.

##### 3.2.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

##### 3.2.6 Detoxification test

The test given in 3.2.6 of Diphtheria Toxoid and 3.2.6 of Tetanus Toxoid shall apply.

##### 3.2.7 Potency test

The test given in 3.2.7 of Diphtheria Toxoid and 3.2.7 of Tetanus Toxoid shall apply.

##### 3.2.8 Identity test

The test given in 3.2.8 of Diphtheria Toxoid and 3.2.8 of Tetanus Toxoid shall apply.

### **4. Storage and expiry date**

The expiry date shall be two years.

**5. Other requirements**

Information to be provided in package insert and other labeling

Recommended human dose and route of administration, as follows:

For primary immunization, usually 3 doses of 0.5 mL are given by subcutaneous injection at intervals of 3–8 weeks. To humans aged older than 10 years, a dose of 0.1 mL shall be injected primarily and, if untoward reactions are absent or mild, adequately increased doses shall be used for subsequent injections. For the first booster immunization, usually 0.5 mL is given by subcutaneous injection with an interval of not shorter than 6 months after completion of the primary immunization, (in general, during 12 and 18 months after completion of the primary immunization). To those showing severe reactions at the time of primary immunization, the dose shall be adequately reduced. This precaution shall be taken for the subsequent booster immunization. The dose for those aged older than 10 years shall be no more than 0.1 mL.

## **ADSORBED DIPHTHERIA-TETANUS COMBINED TOXOID**

### **1. Descriptive definition**

“Adsorbed Diphtheria-Tetanus Combined Toxoid” is a liquid product containing "diphtheria toxoid" and "tetanus toxoid," which are prepared by treating diphtheria toxin and tetanus toxin, respectively, with formaldehyde by a method minimally impairing the immunogenicity of the toxins and rendered insoluble by adding aluminium salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

### **2. Production control**

#### **2.1 Source materials**

Requirements given in 2.1 of Diphtheria Toxoid and 2.1 of Tetanus Toxoid shall apply.

#### **2.2 Bulk material**

Requirements given in 2.2 of Diphtheria Toxoid and 2.2 of Tetanus Toxoid shall apply.

#### **2.3 Final bulk**

The bulk material shall be diluted with buffered physiological saline or other suitable medium, mixed and supplemented with aluminium salt. The final bulk shall contain not higher than 50 Lf of diphtheria toxoid per mL and not higher than 20 Lf of tetanus toxoid per mL.

Any Appropriate preservatives and stabilizer may be added.

### **3. Control tests**

#### **3.1 Tests on bulk material**

Tests given in 3.1 of Diphtheria Toxoid and 3.1 of Tetanus Toxoid 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 5.4 to 7.4.

##### **3.2.2 Test for aluminium content**

When the test given in General Tests is applied, the aluminium content shall be no higher than 1.0 mg/mL.

##### **3.2.3 Test for thimerosal content**

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

##### **3.2.4 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%.

##### **3.2.5 Sterility test**

The test given in General Tests shall apply.

##### **3.2.6 Test for freedom from abnormal toxicity**

The test given in General Tests shall apply.

##### **3.2.7 Detoxification test**

The test given in 3.2.6 of Diphtheria Toxoid and 3.2.6 of Tetanus Toxoid shall apply.

##### **3.2.8 Potency test**

The test given in 3.2.8 of Adsorbed Diphtheria Toxoid and 3.2.8 of Adsorbed Tetanus Toxoid shall apply.

#### 3.2.9 Identity test

The test given in 3.2.8 of Diphtheria Toxoid and 3.2.8 of Tetanus Toxoid shall apply on the test sample, which is solubilized with sodium citrate or other suitable reagent.

#### **4. Storage and expiry date**

The expiry date shall be two years.

#### **5. Other requirements**

##### 5.1 Information to be provided in package insert and other labeling

- (1) Caution that the product be rendered homogenous by thorough shaking before use
- (2) Recommended human dose and route of administration, as follows:

For primary immunization, usually 3 doses of 0.5 mL are given by subcutaneous injection with intervals of 3–8 weeks. To humans aged older than 10 years, a dose of 0.1 mL shall be injected primarily and, if untoward reactions are absent or mild, adequately increased doses shall be injected for subsequent inoculations. For booster immunization, usually 0.5 mL is given by subcutaneous injection with the interval of not shorter than 6 months after the completion of the primary immunization (in general, 12–18 months after the completion of the primary immunization). To those showing severe untoward reactions at the primary immunization, the dose shall be adequately reduced. This precaution shall be valid for the subsequent booster immunizations. The dose for those aged older than 10 years shall be no more than 0.1 mL.

# VARICELLA ANTIGEN

## 1. Descriptive definition

“Varicella Antigen” is a colorless clear liquid preparation containing active substances required for skin reactions specific to varicella.

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Strains of virus used for production

Strains of virus approved as adequate for production (hereafter referred to as "virus" in this monograph) shall be used.

#### 2.1.2 Human diploid cells

For cultivation of the virus, passaged human diploid cells, which were previously approved as adequate for vaccine production (hereafter referred to as "cells" in this monograph) and stored below -70°C, shall be used as cell seed. The seed lots shall be subjected to the tests given in 3.1.

#### 2.1.3 Culture media

A cell culture medium suitable for the propagation of the cells shall be used. The cell culture medium can be supplemented with appropriate cell growth-promoting substances and minimal amounts of antibiotics, except penicillin.

A medium suitable for propagation of each strain of virus shall be used. The medium for propagation of the virus can be supplemented with appropriate stabilizer and minimal amounts of antibiotics, except penicillin.

If serum or a fraction thereof is added to the medium as a cell growth-promoting substance, its amount in the final bulk shall be no higher than 50 ng per dose.

### 2.2 Bulk material

#### 2.2.1 Cell culture

Cells derived from a frozen vial of the cell seed shall be cultivated continuously but shall not be passaged more than 30 times. Cells cultivated in 1 lot shall be regarded as an “individual cell culture.”

The individual cell cultures shall be subjected to the tests given in 3.2.

#### 2.2.2 Antigen suspension

Antigen suspension from individual cell cultures shall be harvested and pooled to serve as a “single harvest.” The single harvest shall be subjected to the tests given in 3.3.1.

#### 2.2.3 Inactivation and purification

Inactivation of virus particles shall be conducted by heating a single harvest at 56°C for 30 minutes to make an “inactivated single harvest.”

The inactivated single harvest shall be subjected to the tests given in 3.3.2.

The inactivated single harvests shall be pooled appropriately, purified, and concentrated, if necessary, to make a “bulk material.”

The bulk material shall be subjected to the tests given in 3.4.

## 2.3 Final bulk

The bulk material shall be diluted, if necessary, to make the “final bulk.” Appropriate stabilizer may be added. However, antibiotic shall not be added. The final bulk shall be subjected to the tests given in 3.5.

## 3. Control tests

### 3.1 Tests on cell seed

Cells derived from the cell seed shall be cultivated continuously, and cells passaged at least 30 time shall be subjected to the following tests.

#### 3.1.1 Chromosomal characterization

Metaphase cells shall be prepared from at least 4 individual cell cultures derived from 1 lot of cell seed in an appropriate method and subjected to the test given in 3.1.1.1. Cell seed may be cultivated within additional three passages, if necessary, for the preparation of metaphase cells.

##### 3.1.1.1 Tests on chromosomal abnormalities

###### 3.1.1.1.1 Test on polyploidy

At least 300 cells shall be examined for polyploidy, and frequency of polyploidy shall be less than the permissible level.

###### 3.1.1.1.2 Test on heteroploidy

At least 100 cells shall be examined for heteroploidy, and a frequency of heteroploidy shall be less than the permissible level.

###### 3.1.1.1.3 Test on structural abnormality

At least 100 cells shall be examined for structural abnormality, and a frequency of structural abnormality shall be less than the permissible level.

###### 3.1.1.1.4 Test on chromosome breaks

At least 100 cells shall be examined for chromosome breaks, and a frequency of chromosome breaks shall be less than the permissible level.

###### 3.1.1.1.5 Test on karyotypes

At least 1 cell shall be examined for karyotypes, and shall be normal.

#### 3.1.2 Observation of cell culture

The cells shall be cultivated without being inoculated with virus under the same conditions as the culture of virus for 14 days. No cytopathic change due to extraneous virus shall be detected. During the observation period, cultures discarded due to any nonspecific or incidental reason shall be less than 20% of the total cultures.

#### 3.1.3 Tests in cell cultures

At the end of the observation period above, the culture medium shall be harvested from each vessel, pooled if necessary, and subjected to the tests given in 3.3.1.2.

#### 3.1.4 Freedom from tumorigenicity

At the end of the observation period above, at least 4 cultures of the cells shall be shown to be free from tumorigenicity. Athymic mice (nude nu/nu genotype), or immunosuppressed mice or hamsters shall be used for the test. At least 5 animals shall be given by subcutaneous injection with

more than  $2 \times 10^6$  cells. The animals shall be observed for 28 days. No animal shall show evidence of tumor formation. As control, at least 5 animals shall be similarly injected with more than  $2 \times 10^6$  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.

### 3.2 Tests on individual cell culture

A quantity of 5% of individual cell cultures or a quantity corresponding to 500 mL shall be used as the control cell culture. The control cell culture shall be subjected to the tests given below.

#### 3.2.1 Identity test

The cells shall be shown to be human diploid cells in the test given in 3.1.1.1.5.

#### 3.2.2 Observation of cell culture

The control cell cultures shall be incubated without being inoculated with virus under the same conditions as the culture of virus. No cytopathic change due to extraneous virus shall be detected. During the observation period, cultures discarded due to any nonspecific or incidental reason shall be less than 20% of the total control cell cultures.

#### 3.2.3 Tests in cell cultures

At the end of the observation period above, the culture medium shall be harvested from each vessel, pooled if necessary, and subjected to the tests given in 3.3.1.2.

### 3.3 Tests on antigen suspension

#### 3.3.1 Tests on single harvest

##### 3.3.1.1 Sterility test

Sterility Test, Mycoplasma Test, Mycobacterial Sterility Test given in General Tests shall apply. For the Mycobacterial Sterility Test, a 25-mL portion of the test sample shall be centrifuged and resuspended in 5 mL of physiological saline prior to use.

##### 3.3.1.2 Tests for freedom from extraneous viruses

The tests shall be performed, if necessary, after neutralization of virus with anti-varicella virus serum prepared in animals except monkeys, humans, and cows.

###### 3.3.1.2.1 Inoculation of African green monkey kidney culture cells

A 10-mL portion of the test sample shall be inoculated into an African green monkey kidney cell culture. The inoculated cell cultures shall be incubated and observed for 14 days. No hemadsorption with guinea pig and chicken red blood cells shall be detected. No cytopathic changes due to extraneous viruses shall be detected.

###### 3.3.1.2.2 Inoculation of human tissue culture cells

A 10-mL portion of the test sample shall be inoculated into human diploid cell cultures. The inoculated cell cultures shall be incubated and observed for 14 days. No cytopathic change due to extraneous viruses shall be detected.

#### 3.3.2 Tests on inactivated single harvest

##### 3.3.2.1 Sterility test

The test given in General Tests shall apply.

##### 3.3.2.2 Tests for inactivation

A 10-mL portion of the test sample shall be inoculated into human fetal lung cell cultures. The inoculated cell cultures shall be incubated and observed for 14 days. No cytopathic change due to

varicella virus shall be detected.

### 3.4 Tests on bulk material

#### 3.4.1 Staining test

The test given in General Tests shall apply.

#### 3.4.2 Sterility test

The test given in General Tests shall apply.

#### 3.4.3 Potency test

The bulk material shall be subjected to the tests given in 3.6.5.

### 3.5 Tests on final bulk

#### 3.5.1 Sterility test

The test given in General Tests shall apply.

#### 3.5.2 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.6 Tests on final product

Following tests shall apply to each final lot.

#### 3.6.1 Test for pH

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

#### 3.6.2 Test for protein nitrogen content

When the test given in General Tests is applied, the protein nitrogen content shall be no higher than 20µg/mL.

#### 3.6.3 Sterility test

The test given in General Tests shall apply.

#### 3.6.4 Inactivation test

The test given in 3.3.2.2 shall apply. No evidence of virus growth shall be shown.

#### 3.6.5 Potency test

The potency of the test sample as measured in enzyme immunoassays shall be no less than that of the Reference.

#### 3.6.6 Identity test

The content shall be identified by serological methods.

## 4. Storage and expiry date

The expiry date shall be one year.

## 5. Other requirements

### 5.1 Information to be provided in package insert and other labeling

- (1) Name of the virus strain used for production.
- (2) Name of the cell culture used for the cultivation of virus.
- (3) Names and concentrations of antibiotics or dyes used in the cultivation of virus, if applicable.
- (4) Recommended human dosage and route of administration, as follows:

Usually, 0.1-mL is given by subcutaneous injection once.

# **FREEZE-DRIED LIVE ATTENUATED VARICELLA VACCINE**

## **1. Descriptive definition**

“Freeze-dried Live Attenuated Varicella Vaccine” is a dried product containing attenuated varicella virus (hereafter referred to as "virus" in this monograph). When reconstituted, it becomes a colorless or slightly whitish clear liquid.

## **2. Production control**

### **2.1 Source materials**

#### **2.1.1 Strains of virus used for production**

Strains of virus approved as adequate for production shall be used. The seed lots shall be prepared from the strains and subjected to the tests given in 3.1. Virus contained in the product shall be passaged under the approved culture conditions and shall not be passaged more than 10 times from the original vaccine approved as adequate for the strain for production.

#### **2.1.2 Cell seed**

For cultivation of the virus, passaged human diploid cells approved as adequate for vaccine production (hereafter referred to as "cells" in this monograph) shall be used as cell seed. Cell seed shall be subjected to the tests given in 3.2. Passaged cells shall be stored below -70°C.

#### **2.1.3 Trypsin and Bovine serum**

For cell treatment and cultivation, trypsin and bovine serum confirmed as free from extraneous agents shall be used.

#### **2.1.4 Culture media**

Cell culture medium suitable for propagation of the cells shall be used. The cell culture medium can be supplemented with appropriate cell growth-promoting substances, phenol red at a concentration not higher than 0.002 w/v%, and minimal amounts of antibiotics, except penicillin.

The medium suitable for propagation of each strain of virus shall be used.

The medium for propagation of the virus can be added with phenol red at a concentration not higher than 0.002 w/v%, appropriate stabilizer, and minimal amounts of antibiotics, except penicillin.

If serum or a fraction thereof is added to the medium as a cell growth-promoting substance, its amount in the final bulk shall be no higher than 50 ng per dose.

### **2.2 Bulk material**

#### **2.2.1 Cell culture**

Continuously cultivated cells that are derived from one or a mixture of 2 or more frozen vials of cell seed shall be regarded as an “individual cell culture.”

The cells shall not be more than 30 passages from the cell seed. When the cell cultures are observed before the inoculation of virus, no cytopathic change shall be detected.

The individual cell cultures shall be subjected to the tests given in 3.3.

#### **2.2.2 Virus suspension**

Human diploid cells shall be used for the cultivation of virus. Virus suspensions from individual cell cultures shall be harvested and pooled to serve as the “single harvest.” Appropriate stabilizer can

be added.

The single harvest shall be subjected to the tests given in 3.4.1.

#### 2.2.3 Filtration

The single harvests shall be pooled, centrifuged, and filtered to eliminate cells to make the “virus pool.” The virus pool shall be subjected to the tests given in 3.4.2.

Virus pools shall be pooled to make a “bulk material.”

The bulk material shall be subjected to the tests given in 3.5.

#### 2.3 Final bulk and freeze-drying

The bulk material shall be diluted, if necessary, to make the “final bulk.” Appropriate stabilizer may be added. However, antibiotics shall not be added.

The final bulk shall be dispensed into final containers and freeze-dried.

The final bulk shall be subjected to the tests given in 3.6.

### 3. Control tests

#### 3.1 Tests on virus seed

The tests given in 3.5 and 3.1.1 shall apply. The sterility test given in 3.2.1 shall apply.

##### 3.1.1 Neurovirulence safety test

The test shall be performed in *Macacus* or *Cercopithecus* monkeys shown to be serologically negative for varicella virus. At least 10 monkeys shall be employed in each test. Test sample containing more than  $10^{4.0}$  PFU/mL shall be injected into the thalamic region of each hemisphere at a dose of 0.5 mL and into the cerebellomedullary cistern at a dose of 0.25 mL. The animals shall be observed for 21 days. No signs of paralysis or other neurologic disorders shall be detected, and more than 80% of the animals must survive the observation period. At the end of the observation period, each animal shall be autopsied for histopathological examination of the central nervous system. No evidence of changes due to the presence of any virus or of extraneous microbial agents in the test sample shall be demonstrated.

#### 3.2 Tests on cell seed

##### 3.2.1 Sterility test

Sterility Test, Mycoplasma Test, Mycobacterial Sterility Test given in General Tests shall apply.

##### 3.2.2 Tests for freedom from extraneous viruses

###### 3.2.2.1 Tests in animals

###### 3.2.2.1.1 Inoculation of adult mice

At least 10 mice aged 4–5 weeks shall be inoculated intramuscularly with more than  $10^6$  cells. The animals shall be observed for 4 weeks. No animal shall show evidence of infection with extraneous microbial agents, and more than 80% of the animals must survive the observation period.

###### 3.2.2.1.2 Inoculation of suckling mice

At least 10 suckling mice shall be inoculated intramuscularly within 24 hours after birth with more than  $10^6$  cells. The animals shall be observed for 4 weeks. Any animals that die within 1 day after injection shall be excluded from the analysis. No animal shall show evidence of infection with extraneous microbial agents, and more than 80% of the animals must survive the observation period.

###### 3.2.2.1.3 Inoculation of guinea pigs

At least 5 guinea pigs weighing 300–400 g shall be inoculated intramuscularly with  $2 \times 10^6$  cells. The animals shall be observed for 4 weeks. No animal shall show evidence of infection with extraneous microbial agents, and more than 80% of the animals must survive the observation period.

#### 3.2.2.1.4 Inoculation of rabbits

At least 5 rabbits weighing 1.5-2.5 kg shall be inoculated intramuscularly with more than  $2 \times 10^6$  cells. The animals shall be observed for 4 weeks. No animal shall show evidence of infection with extraneous microbial agents, and more than 80% of the animals must survive the observation period.

#### 3.2.2.2 Tests in cell cultures

A mixture of the culture media of cell seed shall be subjected to the tests given in 3.5.3.2.

#### 3.2.2.3 Inoculation of embryonated chicken eggs

The test sample shall be inoculated at a dose of  $10^5$  cells onto the allantoic cavities of at least 20 embryonated chicken eggs aged 10–11 days. The inoculated eggs shall be observed for 3 days. Any eggs that die within 1 day after injection shall be excluded from the analysis. No change due to extraneous viruses shall be detected, and more than 80% of the eggs must survive the observation period. Furthermore, samples from dead eggs shall be inoculated into at least 10 eggs and observed as given above. No change due to extraneous viruses shall be detected, and more than 80% of the eggs must survive the observation period.

#### 3.2.3 Chromosomal characterization

Cells derived from the cell seed shall be cultivated continuously to the same or higher passage number as the cultures used for the production of virus. At least 4 cultures of the cells shall be subjected to the following tests.

##### 3.2.3.1 Test on polyploidy

At least 300 cells shall be examined for polyploidy, and a frequency of polyploidy shall be less than the permissible level.

##### 3.2.3.2 Test on heteroploidy

At least 100 cells shall be examined for heteroploidy, and a frequency of heteroploidy shall be less than the permissible level.

##### 3.2.3.3 Test on structural abnormality

At least 100 cells shall be examined for structural abnormality, and a frequency of structural abnormality shall be less than the permissible level.

##### 3.2.3.4 Test on chromosome breaks

At least 100 cells shall be examined for chromosome breaks, and a frequency of chromosome breaks shall be less than the permissible level.

##### 3.2.3.5 Test on karyotypes

At least 1 cell shall be examined for karyotypes, and shall be normal.

#### 3.2.4 Freedom from tumorigenicity

At least 4 cultures of cells cultivated as given in 3.2.3 shall be shown to be free from tumorigenicity.

Athymic mice (nude nu/nu genotype), or immunosuppressed mice or hamsters, shall be used for the test. At least 5 animals shall be given by subcutaneous injection with more than  $2 \times 10^6$  cells. The

animals shall be observed for 28 days. No animal shall show evidence of tumor formation. As control, at least 5 animals shall be similarly injected with more than  $2 \times 10^6$  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.

### 3.3 Tests on individual cell cultures

A quantity of 5% of individual cell cultures or a quantity corresponding to 500 mL shall be used as the control cell culture. The control cell culture shall be subjected to the tests given below.

#### 3.3.1 Observation of cell culture

The control cell cultures shall be incubated without being inoculated with virus under the same conditions as the culture of virus. No cytopathic change due to extraneous virus shall be detected. During the observation period, cultures discarded due to any nonspecific or incidental reason shall be less than 20% of the total control cell cultures.

#### 3.3.2 Tests in cell cultures

At the end of observation period above, the culture medium shall be harvested from each vessel, pooled if necessary, and subjected to the tests given in 3.5.3.2.

#### 3.3.3 Test for freedom from hemadsorption

At the end of the observation period above, no hemadsorption with guinea pig red blood cells due to extraneous viruses shall be detected.

### 3.4 Tests on virus suspension

#### 3.4.1 Tests on single harvest

##### 3.4.1.1 Sterility test

Sterility Test, Mycoplasma Test, Mycobacterial Sterility Test given in General Tests shall apply. For the Mycobacterial Sterility Test, a 25-mL portion of the test sample shall be centrifuged and resuspended in 5 mL of physiological saline prior to use.

##### 3.4.1.2 Test for virus content

The test given in 3.7.3 shall apply.

#### 3.4.2 Tests on virus pool

##### 3.4.2.1 Sterility test

The test given in General Tests shall apply.

##### 3.4.2.2 Tests for freedom from extraneous viruses

The tests given in 3.5.3.2 shall apply. In this case, the tests shall be performed, if necessary, after the neutralization of virus with anti-varicella virus serum prepared in animals except monkeys, humans, and cows.

##### 3.4.2.3 Test for virus content

The test given in 3.7.3 shall apply.

### 3.5 Tests on bulk material

#### 3.5.1 Staining test

The test given in General Tests shall apply.

#### 3.5.2 Sterility test

The test given in General Tests shall apply.

#### 3.5.3 Tests for freedom from extraneous viruses

The tests shall be performed after the neutralization of virus, if necessary, following the procedures given in 3.4.2.2.

### 3.5.3.1 Tests in animals

#### 3.5.3.1.1 Inoculation of adult mice

At least 10 mice each, aged 4–5 weeks, shall be inoculated intraperitoneally with 0.5 mL or intracerebrally with 0.03 mL of the test sample. The animals shall be observed for 21 days. No animal shall show evidence of infection with extraneous microbial agents, and more than 80% of the animals must survive the observation period,

#### 3.5.3.1.2 Inoculation of suckling mice

At least 20 suckling mice each, aged less than 24 hours, shall be inoculated intraperitoneally with 0.1mL or intracerebrally with 0.01 mL of the test sample. The animals shall be observed for 14 days. Any mice that die within 1 day of injection shall be excluded from the test. No animal shall show evidence of infection with extraneous microbial agents, and more then 80% of the animals must survive the observation period.

#### 3.5.3.1.3 Intracerebral inoculation of guinea pigs

At least 5 guinea pigs weighing 300–400 g shall be inoculated intracerebrally with 0.1 mL of the test sample. The animals shall be observed for 14 days. No animal shall show evidence of infection with extraneous microbial agents, and more than 80% of the animals must survive the observation period.

### 3.5.3.2 Tests in cell cultures

#### 3.5.3.2.1 Inoculation of African green monkey kidney culture cells

A 10-mL portion of the test sample shall be inoculated into African green monkey kidney cell cultures. The inoculated cell cultures shall be incubated and observed for 14 days. No cytopathic change due to extraneous viruses shall be detected.

#### 3.5.3.2.2 Inoculation of human tissue culture cells

A 10-mL portion of the test sample shall be inoculated into human diploid cell cultures. The inoculated cell cultures shall be observed for 14 days. No cytopathic change due to extraneous viruses shall be detected.

### 3.5.4 Identification test

The inoculated virus in appropriate cell cultures shall show virus-specific cytopathic effects. This cytopathic effect shall be suppressed after neutralization of virus in the test sample with anti-varicella virus serum.

### 3.5.5 Test for virus content

The test given in 3.7.3 shall apply.

## 3.6 Tests on final bulk

### 3.6.1 Sterility test

The test given in General Tests shall apply.

### 3.6.2 Test for virus content

The test given in 3.7.3 shall apply.

## 3.7 Tests on final product

Following tests shall apply to each final lot.

#### 3.7.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### 3.7.2 Sterility test

The test given in General Tests shall apply.

#### 3.7.3 Potency test

The titration of virus performed in suitable cell culture systems shall constitute the measure of potency of the live virus. The potency of the test sample shall be no less than 1,000 PFU per 0.5 mL.

#### 3.7.4 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.7.5 Identity test

Virus in the test sample shall be identified by immunofluorescence assay after propagation of the virus in appropriate cell cultures.

### **4. Storage and expiry date**

Storage temperature shall be 5°C or less. The expiry date shall be one year.

### **5. Other requirements**

#### 5.1 Reconstituent

The reconstituent shall be water for injection.

#### 5.2 Information to be provided in package insert and other labeling

- (1) Name of the virus strain used for production.
- (2) Name of the cell culture used for the cultivation of virus.
- (3) Names and concentrations of antibiotics or dyes used in the cultivation of virus, if applicable.
- (4) Recommended human dosage and route of administration, as follows:

Usually, 0.5 mL is given by subcutaneous injection once.

# TYPHOID-PARATYPHOID VACCINE

## 1. Descriptive definition

“Typhoid-Paratyphoid Vaccine” is a whitish turbid liquid product containing inactivated *Salmonella typhi*, *Salmonella paratyphi A* and *Salmonella Paratyphi B* (hereafter referred to as "bacteria" in this monograph). A product containing inactivated organisms of *S. typhi* only may be manufactured, if necessary.

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Strains of bacteria used for production

Strain Ty-2 of *S. typhi*, strain 41-N-22 of *S. paratyphi A* and strain 41-H-6 of *S. paratyphi B* or other strain with equivalent or higher antigenicity shall be used.

#### 2.1.2 Culture medium used for production

The medium used for the cultivation of bacteria shall not contain any ingredient, which has the possibility of inducing a pronounced allergic reactions in humans.

### 2.2 Bulk material

#### 2.2.1 Suspensions of bacteria

The bacteria shall be cultivated at  $36 \pm 1^\circ\text{C}$  for a period not longer than 24 hours. The cultured bacteria shall be harvested and suspended in physiological saline or other suitable medium. The suspension shall be tested microscopically and by appropriate culture methods. If no contaminant is detected, the suspension shall serve as the single-strain suspension.

#### 2.2.2 Inactivation

The inactivation of bacteria shall be conducted by heating the single-strain suspension at  $56 \pm 1^\circ\text{C}$  for 60 minutes, combining with phenol at 0.5 w/v% immediately after heating, and then holding at  $20\text{--}25^\circ\text{C}$  for an appropriate period. Other suitable methods proved to be capable of inactivating the organisms without any appreciable loss of antigenicity may be used. After inactivation, the single-strain suspension shall serve as the single-strain bulk.

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

### 2.3 Final bulk

The single-strain bulk shall be diluted in buffered physiological saline or other suitable medium. They shall be mixed such as to make the concentration of *S. typhi* to be  $1.0 \times 10^9$ , *S. paratyphi A* to be  $0.25 \times 10^9$  and *S. paratyphi B* to be  $0.25 \times 10^9/\text{mL}$  when determined by the test given in 3.1.1. Phenol shall be added at 0.5 w/v%. The final bulk shall be subjected to the tests given in 3.2.

## 3. Control tests

### 3.1 Tests on single-strain bulk

#### 3.1.1 Test for content of bacteria

The test for optical density given in General Tests shall apply. The optical density of a test sample containing  $1.0 \times 10^9$  of each bacteria per mL shall correspond to 10 opacity units.

#### 3.1.2 Staining test

The test given in General Tests shall apply.

#### 3.1.3 Sterility test

The test given in General Tests shall apply.

## 3.2 Tests on final bulk

### 3.2.1 Sterility test

The test given in General Tests shall apply.

### 3.2.2 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.2.3 Test for toxicity to mouse weight gain

The test given in 3.3.6 shall apply.

## 3.3 Tests on final product

Following tests shall apply to each final lot.

### 3.3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

### 3.3.2 Test for phenol content

The test given in General Tests shall apply.

### 3.3.3 Test for content of bacteria

When the test given in General Tests is applied, the opacity shall be no higher than 15 units.

### 3.3.4 Sterility test

The test given in General Tests shall apply.

### 3.3.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.3.6 Test for toxicity to mouse weight gain

The test sample shall be given by intraperitoneal injection into at least 5 mice aged about 5 weeks at a dose of 0.5 mL. The inoculated animals shall be observed for 3 days. The average body weight 3 days after injection shall be no less than that at the time of injection. No animal shall show any abnormal sign during the observation period.

### 3.3.7 Potency test

Potency shall be determined in mice by the intraperitoneal challenge method using live *S. typhi* bacteria suspended in a mucin solution.

#### 3.3.7.1 Materials

The test sample and strain 63 of *S. typhi* for challenge shall be used. The diluent for the test sample shall be 0.013 mol/L phosphate-buffered sodium chloride solution (pH 7.0). Strain 63 of *S. typhi* for challenge, cultivated at  $36 \pm 1^\circ\text{C}$  for approximately 18 hours, shall be made into a suspension in the 5 w/v% mucin solution. The suspension shall contain live bacteria of approximately 1,000 LD<sub>50</sub> per 0.5 mL as determined by intraperitoneal injection into mice. This suspension shall serve as the challenge suspension.

#### 3.3.7.2 Test procedures

The test sample shall be diluted serially into three levels at a 10-fold or other appropriate logarithmic interval. Each dilution shall be given by intraperitoneal injection at a dose of 0.5 mL into at least 10 mice aged 4 weeks. After an interval of 10–14 days from immunizing injection, the challenge suspension shall be given by intraperitoneal injection into the animals at a dose of 0.5 mL. The animals shall be observed for 3 days.

Serial dilutions of three or more levels of the challenge suspension shall be given by intraperitoneal injection into at least 10 mice each to titrate the virulence. The LD<sub>50</sub> per 0.5 mL of the challenge suspension shall be approximately 1,000.

#### 3.3.7.3 Criterion for judgment

The test sample shall contain no less than 200 ED<sub>50</sub> per mL upon statistical analysis.

### 3.3.8 Identity test

The test shall be conducted by the tube agglutination method using immune serum for *S. typhi*.

## 4. Storage and expiry date

The expiry date shall be 18 months.

## 5. Other requirements

### 5.1 Modification of the proper name

Product containing *S. typhi* only shall be named “Typhoid Vaccine.”

### 5.2 Information to be provided in package insert and other labeling

- (1) Names of the strains of bacterial contained in the product
- (2) Caution that the product be rendered homogenous by thorough shaking before use
- (3) Recommended human dose and route of administration, as follows:

For primary immunization, usually 3 doses of 0.25 mL to infants aged 36–48 months and of 0.4 mL to others at intervals of 5–10 days by subcutaneous injection to the lateral skin of the upper arm.

For individuals whose health is compromised or those with a tendency to show a severe untoward reaction to the product, 3 doses of 0.1 mL shall be inoculated intracutaneously, rather than subcutaneously, at intervals of 5–10 days. For urgent primary immunization, the intervals can be shortened to 3 days. For booster injection, the usual dosage is 0.4 mL by subcutaneous injection or 0.1 mL by intracutaneous injection. For individuals whose health is compromised and those with a tendency to show a severe adverse reaction to the product, booster injection shall be via intracutaneous injection.

## PURIFIED TUBERCULIN

### 1. Descriptive definition

“Purified Tuberculin” is a white dried product containing the active substance causing specific cutaneous reactions to tuberculosis obtained from the culture filtrate of *Mycobacterium tuberculosis*. When reconstituted, it becomes a colorless clear liquid.

### 2. Production control

#### 2.1 Source materials

##### 2.1.1 Strain of *M. tuberculosis* used for production

Strain Aoyama B of *M. tuberculosis* sourced from the National Institute of Infectious Diseases shall be used.

##### 2.1.2 Culture media used for production

Sauton's medium or other suitable protein-free medium shall be used for production.

For maintenance of the strain, Ogawa's medium may be used, but at least 2 successive transfers shall be made in the medium for production before making a culture for production.

#### 2.2 Powdered bulk

##### 2.2.1 Culture filtrate

###### 2.2.1.1 Cultivation and killing of *M. tuberculosis*

The strain for production shall be inoculated onto the culture medium for production, which shall be cultivated for about 6 weeks. During or on the completion of cultivation, any culture flasks with a submerged pellicle or showing abnormal growth or signs of contamination shall be discarded. After the completion of cultivation, the cultures shall be sterilized by steaming at 100°C for 60 minutes.

###### 2.2.1.2 Filtration

The sterilized cultures shall be pooled, freed from pellicles and bacterial mass, and then filtered to remove bacteria. The filtrate shall be stored at 5°C or less (avoiding freezing) to serve as the culture filtrate.

The test sample of the culture shall be subjected to the tests given in 3.1.

##### 2.2.2 Purification

Purification processes shall be conducted at 5°C or less.

###### 2.2.2.1 Concentrated filtrate

The culture filtrate shall be concentrated to no more than about 1/30 of the volume of the original filtrate by ultrafiltration. The concentrate shall be dissolved in 0.016 mol/L phosphate buffer (pH 7.2) or other suitable medium and subjected again to ultrafiltration to serve as the concentrated filtrate.

###### 2.2.2.2 Desalted concentrated filtrate

An equal amount of a saturated solution of ammonium sulfate (pH 7.2) shall be added to the concentrated filtrate. The precipitate shall be collected and dissolved in 0.016 mol/L phosphate buffer (pH 7.2) or other suitable solution. The addition of the saturated solution of ammonium sulfate and collection and dissolving of the sediment shall be repeated at least twice.

The final precipitate shall be dissolved in a suitable buffered solution, freed from ammonium sulfate by gel filtration, and sterilized by filtration. The sterilized filtrate shall serve as the desalted concentrated bulk.

The desalted concentrated bulk shall be subjected to the tests given in 3.2.

#### 2.2.3 Freeze drying

The desalted concentrated bulk shall be freeze-dried to serve as a powdered bulk. The powdered bulk shall be stored at less than 10°C, avoiding moisture. The powdered bulk shall be subjected to the tests given in 3.3.

#### 2.3 Final bulk

An adequate amount of the powdered bulk shall be precisely measured and dissolved in 0.5w/v% lactose solution such as to prepare the potency required in 5.1.

#### 2.4 Final product

The final bulk shall be dispensed in final containers in 1.0 mL volumes and freeze-dried. The volume shall be 0.5 mL for the final products given in 5.1 (4) and 5.1 (7). After the completion of drying, the containers shall be hermetically sealed under reduced pressure or after the residual air is replaced with dried air or nitrogen gas to serve as the final product.

### 3. Control tests

#### 3.1 Tests on culture filtrate

The sterility test and test for mycobacterial sterility given in General Tests shall apply. The volume of the test sample subjected to the test for mycobacterial sterility shall be 2 mL.

#### 3.2 Tests on desalted concentrated bulk

##### 3.2.1 Sterility test

Sterility Test and Mycobacterial Sterility Test given in General Tests shall apply. The volume of test sample to be subjected to the test for mycobacterial sterility shall be 2 mL.

##### 3.2.2 Test in animals for freedom from live mycobacteria

The test sample shall be diluted two-fold in 1.7 w/v% sodium chloride solution.

The dilution shall be given by intraperitoneal injection into at least 5 guinea pigs weighing 300–400 g at a dose of 5 mL. The inoculated animals shall be observed for 6 weeks. No animal shall show any abnormal sign during the observation period.

At the end of the observation period, a 2 µg/mL solution of Standard Purified Tuberculin (hereafter referred to as "Standard" in this monograph) shall be given by intracutaneous injection into the back of animals at a dose of 0.1 mL. The animals shall be observed for 24 hours. No animal shall show local redness of a diameter larger than 9mm. No animal shall show any tuberculous lesion when examined by autopsy.

#### 3.3 Tests on powdered bulk

##### 3.3.1 Test for moisture content

Moisture content shall be no higher than 5.0% when tested according to the test given in General Tests.

##### 3.3.2 Test for protein nitrogen content

When the test given in General Tests is applied, the protein nitrogen content shall be no less than 10%.

##### 3.3.3 Test for sugar content

When the test given in General Tests is applied, the sugar content shall be no higher than 5%.

##### 3.3.4 Sterility test

The test sample shall be dissolved in the solution given in 5.2 at 1 mg/mL, and the test given in General Tests shall apply.

### 3.3.5 Potency test

The test shall be conducted using the primary and secondary tests in sensitized guinea pigs, as well as a confirmatory test in humans. The solvent and diluent for the test sample and the Standard shall be that given in 5.2.

#### 3.3.5.1 Sensitized guinea pigs

Killed and dried bacteria of the Aoyama B strain of *M. tuberculosis* shall be suspended in liquid paraffin at 0.1 mg/mL.

The suspension shall be given by intramuscular injection at a dose of 0.25 mL into the thighs of both legs of white female guinea pigs weighing 300–400 g. Six weeks after the injection, the Standard shall be given by intracutaneous injection at concentrations of 2 and 0.5 µg/mL into the left and right shoulders, and the sizes of local indurations shall be measured after 24 hours.

When the difference in diameter between the reaction due to the 2 µg/mL solution and that due to the 0.5 µg/mL solution is more than 4 mm, the animal can be used as a sensitized guinea pig.

#### 3.3.5.2 Primary test

The test sample shall be dissolved at 2 µg/mL to serve as the test solution. The Standard shall be made into 5 suitable logarithmic serial dilutions.

Each of the test dilution and the dilutions of the Standard shall be given by intracutaneous injection at a dose of 0.1 mL into 6 sensitized guinea pigs at 6 sites of the back. The site of injection shall be selected by the Latin square method. The induration of each site shall be measured 24 hours after injection.

The induration caused by the test dilution shall correspond to that caused by the dilution of the Standard containing 1.0–5.0 µg/mL upon statistical analysis.

#### 3.3.5.3 Secondary test

Each of the test sample and the Standard shall be made to three logarithmic serial dilutions. The dilutions of the test sample shall be made to cause reactions comparable to those of the Standard from the results of the primary test. The dilutions shall be given by intracutaneous injection into 6 sensitized guinea pigs in the same manner as the primary test. The reactions shall be measured 24 hours after injection.

The relative potency of the test sample to the Standard shall be determined by statistical analysis of test results.

#### 3.3.5.4 Confirmatory test

The Standard shall be diluted to a concentration of 0.5 µg/mL (hereafter referred to as "standard dilution" in this monograph). The test sample shall be made equal dilution with relative potency in guinea pigs of the Standard (hereafter referred to as "test dilution" in this monograph).

Tuberculin-positive individuals, who have never shown strong reaction, each of which consists of the enough number for statistical analysis, shall be injected with 0.1 mL doses of standard dilution on the one arm and test dilution on the other at the inner side of each of the forearms. The sites of injections shall be randomly exchanged between right and left for arms from one person to the next. The test shall be performed by a double-blind method. About 48 hours after injection, the local reactions shall be examined and the strength of reactions of both sides shall be compared each other by the size of redness.

The concentration of the test sample with an equivalent potency to that of the standard dilution shall be calculated by statistical analysis of test results, upon individuals showing no less than 10mm in mean diameters of major and minor axes.

### 3.3.6 Test for sensitizing effect

The test sample shall be made into a solution with potency equal to that of a 100 µg/mL dilution of the Standard from the results obtained in the tests given in 3.3.5. The 100 µg/mL dilution of the Standard shall be used as the control.

The test solution shall be given by intracutaneous injection into at least 3 guinea pigs weighing 300–400 g in 3 times of 0.1 mL doses with intervals of 5 days. With an interval of 15 days after the last injection, the test solution shall be given by intracutaneous injection at a dose of 0.1 mL. The local reactions shall be examined 24 and 48 hours after injection. The injections of the control solution shall be made in the same manner.

The reactions to the test solution shall be not stronger than those to the control solution.

#### 3.4 Tests on final product

Following tests shall apply to each final lot.

##### 3.4.1 Test for moisture content

Moisture content shall be no higher than 5.0% when tested according to the test given in General Tests.

##### 3.4.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 7.2 to 7.5.

##### 3.4.3 Test for sugar content

When the test given in General Tests is applied with a 0.01 w/v% Standard solution of lactose as the Standard, the contents of each container shall be  $5.00 \pm 0.25$  mg. The value shall be  $2.50 \pm 0.13$  mg for the final product given in 5.1(4) and (7).

##### 3.4.4 Test for phenol content

The test given in General Tests shall apply.

##### 3.4.5 Sterility test

The test given in General Tests shall apply.

##### 3.4.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

##### 3.4.7 Potency test

The test sample shall be prepared at a concentration of 0.5–2.0 µg/mL according to the description on the label. The Standard dissolved at the same concentration as the test sample shall serve as the Standard solution.

Each of the test solution and the Standard solution shall be given by intracutaneous injection at a dose of 0.1 mL into at least 5 sensitized guinea pigs in 3.3.5.1 on both the left and right sides of the back. The Standard solution shall be injected at 4 sites on one side and the test solution at the same sites on the other side. One site on one side of a guinea pig shall be injected with the test solution made from one final container.

The potency of the test solution shall be equal to that of the Standard solution upon statistical analysis.

##### 3.4.8 Identity test

The test shall be conducted by intracutaneous injection into sensitized guinea pigs.

#### 4. Storage and expiry date

The expiry date shall be three years.

#### 5. Other requirements

### 5.1 Labeling

The type of the product shall be selected from the following list and given on the label:

- (1) For general diagnosis (5 µg)  
Contents equivalent to 5 µg of the Standard
- (2) For general diagnosis (2.5 µg)  
Contents equivalent to 2.5 µg of the Standard
- (3) For general diagnosis (1 µg)  
Contents equivalent to 1 µg of the Standard
- (4) For general diagnosis (1 dose)  
Contents equivalent to 0.25 µg of the Standard
- (5) For general diagnosis (in humans with severe untoward reactions)  
Contents equivalent to 0.2 µg of the Standard
- (6) For confirmatory diagnosis  
Contents equivalent to 10 µg of the Standard
- (7) For confirmatory diagnosis (1 dose)  
Contents equivalent to 2.5 µg of the Standard

### 5.2 Reconstituent

The reconstituent shall be as follows:

The volume of solution for 5.1(1) shall be 11 mL; for (2) 6 mL; and for (3), (5) and (6) 3 mL; and for (4) and (7) 0.5 mL, respectively.

Sodium monohydrogen phosphate 15.28 g

Potassium dihydrogen phosphate 1.45 g

Sodium chloride 4.80 g

Phenol 5.0 g

The ingredients shall be dissolved in water for injection to a volume of 1,000 mL, which shall be sterilized by filtration.

For the product of (4) and (7), phenol can be removed.

# SMALLPOX VACCINE

## 1. Descriptive definition

“Smallpox Vaccine” is a whitish or grayish turbid liquid product containing live vaccinia virus (hereafter referred to as "virus" in this monograph).

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Strains of virus

The strains of virus approved for production shall be used.

#### 2.1.2 Animals for production

Calves or sheep shall be used.

The animals for production shall be observed under quarantine for at least 14 days prior to inoculation with virus. They shall be free from any signs of illness during the observation period and shall be negative when tested by cutaneous reaction with tuberculin and by agglutination of the serum with *Brucellae*.

### 2.2 Inoculation

The skin of the animal shall be disinfected, scarified, and inoculated with virus. After the inoculation, the surface of the inoculation site can be treated with appropriate antibiotics.

### 2.3 Bulk material

The vaccinal material after virus inoculation (hereafter referred to as "bulk" in this monograph) shall be harvested.

Before harvesting the bulk, the inoculation site shall be washed repeatedly to remove residual antibiotics and the animal shall be killed by exsanguination. After harvesting the bulk, the animal shall be examined by autopsy. If evidence of any infectious disease other than vaccinia is found, the bulk from that animal shall be discarded.

### 2.4 Final bulk

The bulk material shall be suspended in a 50 w/v% glycerin solution or other suitable medium, triturated, and freed from tissue fragments by centrifugation or other suitable method to serve as the final bulk.

Phenol shall be added at a concentration not higher than 0.5 w/v%.

Appropriate stabilizer may be added. No antibiotics shall be added.

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

## 3. Control tests

### 3.1 Tests on final bulk

#### 3.1.1 Test for total bacterial content

The test given in 3.2.1 shall apply.

#### 3.1.2 Test for freedom from *Escherichia coli*

The test given in 3.2.2 shall apply.

#### 3.1.3 Test for freedom from *hemolytic streptococci*

The test given in 3.2.3 shall apply.

#### 3.1.4 Test for freedom from *staphylococci*

The test given in 3.2.4 shall apply.

### 3.1.5 Test for freedom from *Bacillus anthracis*

The test given in 3.2.5 shall apply.

### 3.1.6 Test for freedom from pathogenic *Clostridia*

The test given in 3.2.6 shall apply.

## 3.2 Tests on final product

The final product shall be subjected to the tests given below. The tests given in 3.2.2, 3.2.3, 3.2.4, 3.2.5, and 3.2.6 are usually substituted by those given in 3.1.2, 3.1.3, 3.1.4, 3.1.5, and 3.1.6.

### 3.2.1 Test for total bacterial content

The test sample shall be diluted 20-fold with physiological saline.

The dilution shall be dispensed into no less than 5 Petri dishes at a volume of 1 mL and mixed with thioglycolate-agar medium, which has been melted and cooled to about 45°C. The plates shall be solidified and incubated at  $25 \pm 1^\circ\text{C}$  for 48 hours, then at  $31 \pm 1^\circ\text{C}$  for 48 hours, and further at  $36 \pm 1^\circ\text{C}$  for 24 hours. After incubation, the number of colonies appearing on the plates shall be counted and the mean number per plate shall be calculated. The mean number multiplied by 20 shall be taken as the total bacterial content in 1 mL of the test sample. This number shall be less than 500.

### 3.2.2 Test for freedom from *Escherichia coli*

The test sample shall be diluted 100-fold with physiological saline. The dilution shall be inoculated into at least 3 tubes of EC medium at a dose of 1 mL. The tubes shall be incubated at  $44.5 \pm 0.2^\circ\text{C}$  for 48 hours. No gas formation shall be observed.

### 3.2.3 Test for freedom from *hemolytic streptococci*

The test sample shall be diluted 100-fold in buffered sodium chloride-peptone solution.

The dilution shall be dispensed into at least 3 Petri dishes at a volume of 1 mL, and mixed with blood agar medium. The plates shall be solidified and incubated at  $37 \pm 1^\circ\text{C}$  for 48 hours. If colonies appearing to be of beta-hemolytic *streptococci* are detected, and *streptococci* are observed on the Gram-stained smears, the subcultures shall be subjected to the serological test of Lancefield. In this test, no colony shall be identified to belong to group A *streptococcus*.

### 3.2.4 Test for freedom from *staphylococci*

The test sample shall be diluted 10-fold in brain-heart-infusion medium.

The dilution shall be inoculated at a dose of 0.1 mL onto at least 3 plates of blood agar medium and incubated at  $37 \pm 1^\circ\text{C}$  for 48 hours. If colonies of *staphylococci* are detected, and *staphylococci* are observed on Gram-stained smears, the colonies shall be subcultured in brain-heart-infusion medium and subjected to the coagulase test. In this test, no colony shall be identified to be a coagulase-positive *staphylococcus*.

If blood agar medium is not suited for the test, mannitol salt agar medium shall be used.

### 3.2.5 Test for freedom from *Bacillus anthracis*

The test sample shall be inoculated at a dose of 0.1 mL onto at least 5 plates of nutrient agar media, and incubated at  $37 \pm 1^\circ\text{C}$  for 48 hours.

No colonies of spore-bearing Gram-positive large rods sensitive to gammaphage shall be observed.

### 3.2.6 Test for freedom from pathogenic *Clostridia*

The test sample shall be inoculated into at least 10 tubes of 15 mL of fluid thioglycollate medium I at a dose of 0.15 mL. The inoculated tubes shall be heated at  $64.5 \pm 0.5^\circ\text{C}$  for 1 hour and incubated at  $37 \pm 1^\circ\text{C}$  for 7 days. If any growth is detected, the positive culture shall be transferred to plates of Columbian agar medium and Zeissler's blood agar medium containing glucose. The plates shall be incubated anaerobically. If any colony of *Clostridium* appears, it shall be transferred to

cooked-meat medium and the growth shall be tested in animals. If animals show no sign of infection or intoxication with *Cl. tetani*, *Cl. botulinum*, or other pathogenic *Clostridium*, the test sample is judged to be acceptable. In the animal tests, at least 2 guinea pigs weighing between 300 and 400 g and at least 5 mice aged 4 weeks shall be used for the culture from each colony. The culture fluid shall be given by intramuscular injection into guinea pigs at a dose of 0.5 mL and into mice at a dose of 0.2 mL, each mixed with 0.1 mL of 4 w/v% calcium chloride solution. The inoculated animals shall be observed for 7 days.

### 3.2.7 Potency test

The test shall be conducted by measuring pock-forming units on the chorioallantoic membrane of developing chick embryos.

#### 3.2.7.1 Materials

The test sample and Reference Smallpox Vaccine (hereafter referred to as the "Reference" in this monograph) shall be used. The diluent shall be 0.0067 mol/L phosphate-buffered sodium chloride solution (pH 7.6) or an equivalent phosphate buffer containing 0.2 w/v% gelatin.

#### 3.2.7.2 Test procedures

The test sample and Reference shall each be diluted serially into at least three suitable logarithmic levels (hereafter referred to as "test dilutions" and "reference dilutions" in this monograph).

In at least 10 chick embryos aged 11–12 days, incubation shall be performed with each dilution. A dose of 0.1 mL of the dilution shall be inoculated onto the chorioallantoic membrane of each egg, which has been provided with an artificial air sac, and the eggs shall be incubated at  $36 \pm 1^\circ\text{C}$  for 48–72 hours. The dilution of the test sample and Reference yielding no less than 10 easily countable pocks per membrane in most membranes shall be chosen for the calculation of pocks.

The mean number of pocks per membrane and its dilution factor shall be used to calculate the number of pock-forming units per mL of the test sample and the Reference. The number for the Reference shall be approximately that prescribed for its label.

#### 3.2.7.3 Criterion for judgment

Pock-forming units per mL of the test sample shall be no less than  $10^{7.7}$ .

### 3.2.8 Identity test

The test shall be conducted by pock formation on the chorioallantoic membrane of eggs and by its neutralization with anti-vaccinia immune serum.

## 4. Storage and expiry date

### 4.1 Storage

Storage temperature shall be  $-15^\circ\text{C}$  or less and  $5^\circ\text{C}$  or less before and after delivery from warehouse, respectively.

### 4.2 Expiry date

The expiry date shall be one year. Expiry date from the delivery from warehouse shall be 3 months.

## 5. Other requirements

### 5.1 Final product dispensed in glass capillary

The description of contents on the label for the final product dispensed in glass capillaries shall be, for example, "5 capillaries of 10 doses," where one dose shall be approximately 0.01 mL.

## 5.2 Labeling

The date of issue

## 5.3 Information to be provided in package insert and other labeling

- (1) Name of virus strain used for production.
- (2) Recommended human dose and route of administration, as follows:

Multiple pressure method:

A small amount of vaccine is spread on the extended skin of the site to be vaccinated and a series of strong pressures shall be made tangentially to the skin with a multiple pressure needle.

Bleeding shall be avoided.

For primary vaccination, 5–10 strokes within an area of 3 mm in diameter shall be employed.

For secondary vaccination, 15–20 strokes in an area of 3–5 mm in diameter shall be employed.

Vaccination shall be done at a single site.

## **FREEZE-DRIED SMALLPOX VACCINE**

### **1. Descriptive definition**

“Freeze-dried Smallpox Vaccine” is a freeze-dried product containing live vaccinia virus (hereafter referred to as "virus" in this monograph). When reconstituted, it becomes a whitish or grayish turbid liquid.

### **2. Production control**

#### 2.1 Source materials

##### 2.1.1 Strains of virus

Requirements given in 2.1.1 of Smallpox Vaccine shall apply.

##### 2.1.2 Animals for production

Requirements given in 2.1.2 of Smallpox Vaccine shall apply.

#### 2.2 Inoculation

Requirements given in 2.2 of Smallpox Vaccine shall apply.

#### 2.3 Bulk material

Requirements given in 2.3 of Smallpox Vaccine shall apply.

#### 2.4 Final bulk

The bulk material shall be triturated and suspended in a suitable buffered solution. The suspension shall be centrifuged or treated by appropriate procedures. A suitable stabilizing agent shall be added to the suspension to serve as the final bulk, which shall then be dispensed and freeze-dried. No antibiotic shall be added. Phenol may be used to make a concentration not higher than 0.5 w/v% when reconstituted according to the instruction given on the label.

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

### **3. Control tests**

#### 3.1 Tests on final bulk

Requirements given in 3.1 of Smallpox Vaccine shall apply.

#### 3.2 Tests on final product

Following tests shall apply to each final lot.

The tests 3.2.3, 3.2.4, 3.2.5, 3.2.6 and 3.2.7 are usually substituted by the tests given in 3.1.2, 3.1.3, 3.1.4, 3.1.5 and 3.1.6 of Smallpox Vaccine, respectively.

##### 3.2.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

##### 3.2.2 Test for total bacterial content

Requirements given in 3.2.1 of Smallpox Vaccine shall apply.

##### 3.2.3 Test for freedom from *Escherichia coli*

Requirements given in 3.2.2 of Smallpox Vaccine shall apply.

##### 3.2.4 Test for freedom from *hemolytic streptococci*

Requirements given in 3.2.3 of Smallpox Vaccine shall apply.

##### 3.2.5 Test for freedom from *staphylococci*

Requirements given in 3.2.4 of Smallpox Vaccine shall apply.

##### 3.2.6 Test for freedom from *Bacillus anthracis*

Requirements given in 3.2.5 of Smallpox Vaccine shall apply.

3.2.7 Test for freedom from pathogenic *Clostridia*

Requirements given in 3.2.6 of Smallpox Vaccine shall apply.

3.2.8 Potency test

Requirements given in 3.2.7 of Smallpox Vaccine shall apply.

3.2.9 Stability test

The test given in 3.2.8 shall be applied to the test sample kept at  $37 \pm 1^\circ\text{C}$  for 4 weeks.

Pock-forming units per mL of the test sample shall be no less than 1/10 the value before warming and shall be no less than  $10^{7.7}$ .

3.2.10 Identity test

Requirements given in 3.2.8 of Smallpox Vaccine shall apply.

**4. Storage and expiry date**

4.1 Storage

Storage temperature shall be  $5^\circ\text{C}$  or less and  $10^\circ\text{C}$  or less before and after delivery from warehouse, respectively.

4.2 Expiry date

The expiry date shall be two years after potency test and one year after delivery from warehouse.

**5. Other requirements**

5.1 Reconstituent

The reconstituent shall contain an appropriate concentration of glycerin or other agent to make the solution viscous. The solution shall contain no antibiotics. The volume of the solution shall be 0.01 mL for one dose.

5.2 Labeling

Requirements given in 5.2 of Smallpox Vaccine shall apply.

5.3 Information to be given in package insert and other labeling

Requirements given in 5.3 of Smallpox Vaccine shall apply, in which "vaccine" shall read "reconstituted vaccine."

# SMALLPOX VACCINE PREPARED IN CELL CULTURE

## 1. Descriptive definition

“Smallpox Vaccine Prepared in Cell Culture” (hereafter referred to as "vaccine" in this monograph) is a reddish clear liquid product containing live vaccinia virus (hereafter referred to as "virus" in this monograph).

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Strain of virus

LC16mO strain is a temperature-sensitive and low-virulent strain originated from Lister strain with low-temperature cell culture. Vaccine strain, LC16m8 strain, is obtained by plaque cloning after three passages of the LC16mO strain in cell culture. The master seed of the virus shall be prepared by 5 passages under specified conditions from the original LC16m8 strain, which is approved to be adequate for production.

The strain of virus shall be prepared from the master seed using primary rabbit kidney cell culture.

#### 2.1.2 Animals used for production

Kidney tissues for the production of virus shall be taken from healthy specific pathogen-free (hereafter referred to as "SPF" in this monograph) rabbits aged 1 to 3 weeks. The animals shall be kept in a quarantine room for more than 7 days prior to sacrifice and shall not show any abnormal findings including fever. At autopsy, they shall have no sign of salmonellosis, tuberculosis, pseudotuberculosis and myxomatosis, and the kidneys of the animals shall not show any pathological lesions prohibiting their use for production.

#### 2.1.3 Culture media

The growth medium may contain suitable cell growth factor(s), phenol red not higher than 0.002 w/v%, and minimal amounts of antibiotics. Penicillin shall not be used. If serum or its fraction is added as the growth factor, an appropriate treatment shall be conducted so that the content of albumin in the final bulk shall be no higher than 50 ng/dose.

The maintenance medium for virus-infected cells may contain phenol red no higher than 0.002 w/v%, appropriate stabilizer and minimal amounts of antibiotics. Heterologous serum or its fraction and penicillin shall not be added.

### 2.2 Bulk material

#### 2.2.1 Cell cultures

Kidneys from SPF rabbits shall be digested with an adequate protease, and the primary rabbit kidney cells shall be cultured in an adequate medium. Kidneys from SPF rabbits may be pooled, and cell cultures prepared by one processing shall be regarded as an individual cell culture.

Prior to the inoculation of virus, cell cultures shall be examined microscopically. No cytopathic change shall be detected.

The individual cell cultures shall be subjected to the tests given in 3.1.

#### 2.2.2 Virus cultivation and harvest

Cell cultures shall be inoculated with virus and, after virus propagation under adequate conditions, infected cells shall be harvested. The bulk material shall be prepared by releasing viruses from infected cells by appropriate treatment and clarifying virus suspension by centrifugation or

other procedures.

The bulk material shall be subjected to the tests given in 3.2.

### 2.3 Final bulk

The bulk material shall be mixed and, if necessary, diluted to make the final bulk. Appropriate stabilizer may be added. Antibiotics shall not be added.

The final bulk shall be subjected to the tests given in 3.3.

## 3. Control tests

### 3.1 Tests on cell cultures for production

A quantity of 10% of individual cell cultures shall serve as control and shall be subjected to the tests given below.

#### 3.1.1 Incubation and inspection

The control cell culture shall be incubated without virus-inoculation under the same condition as the virus-inoculated culture for 7 days. No cytopathic changes due to extraneous viruses shall be detected. During the observation period, cultures discarded due to any non-specific or incidental reason shall be no more than 20% of the total control cell culture.

#### 3.1.2 Tests for freedom from extraneous viruses

At the end of the observation period above, the medium shall be harvested from each vessel of the control cell culture, pooled if necessary, and subjected to the tests given below.

##### 3.1.2.1 Tests in animals

###### 3.1.2.1.1 Test in adult mice

The test sample shall be given by intraperitoneal injection at a dose of 0.5 mL or intracerebrally at a dose of 0.03 mL into at least 10 mice aged 4 to 5 weeks and the animals shall be observed for 21 days.

No animal shall show signs of infection with extraneous pathogens, and not fewer than 80% of the animals shall survive.

###### 3.1.2.1.2 Test in suckling mice

The test sample shall be given by intraperitoneal injection at a dose of 0.1 mL or intracerebrally at a dose of 0.01 mL into at least 20 suckling mice aged 24 hours. The inoculated animals shall be observed for 14 days. No animal shall show signs of infection with extraneous pathogens, and at least 80% of the animals shall survive.

###### 3.1.2.1.3 Intraperitoneal test in guinea pigs

The test sample shall be given by intraperitoneal injection at a dose of 5.0 mL into at least 5 guinea pigs weighing between 300 and 400 g. The inoculated animals shall be observed for 42 days. No animal shall show signs of infection with *Mycobacterium tuberculosis*, and at least 80% of the animals shall survive.

###### 3.1.2.1.4 Intracerebral test in guinea pigs

The test sample shall be given by intracerebral injection at a dose of 0.1 mL into at least 5 guinea pigs weighing between 300 and 400 g. The inoculated animals shall be observed for 14 days. No animal shall show signs of infection with extraneous pathogens, and at least 80% of the animals shall survive.

###### 3.1.2.1.5 Test in rabbits

The test sample shall be given by intradermal injection at multiple sites at a dose of 1.0 mL/animal or subcutaneously at 9 mL/animal into at least 5 rabbits weighing between 1.5 and 2.5 kg. The inoculated animals shall be observed for 35 days. No animal shall show signs of infection with

extraneous pathogens, and at least 80% of the animals shall survive.

### 3.1.2.2 Tests in cell cultures

#### 3.1.2.2.1 Test in rabbit kidney cell cultures

A 10-mL portion of the test sample shall be inoculated into rabbit kidney cell cultures. The inoculated cultures are incubated at  $35 \pm 1^\circ\text{C}$  and observed for 14 days. The ratio of volume of the test sample to the maintenance medium of cell cultures shall be within 1:1 to 1:3 and the area of cell sheet shall be at least  $3 \text{ cm}^2/\text{mL}$  of the test sample. At the termination of the observation period, cell cultures shall be harvested by freeze-thawing and inoculated into other rabbit kidney cell cultures. These cultures shall be incubated and observed for 14 days. No cytopathic change due to extraneous viruses shall be detected. At the termination of the observation period, the cultures shall be tested by the addition of guinea pig and chicken erythrocytes. No hemadsorption shall be detected.

### 3.2 Tests on bulk material

#### 3.2.1 Sterility test

Sterility Test, Mycoplasma Test, Mycobacterial Sterility Test given in General Tests shall apply.

### 3.3 Tests on final bulk

#### 3.3.1 Sterility test

Sterility test and Mycoplasma test given in General Tests shall apply.

#### 3.3.2 Marker test

##### 3.3.2.1 Test for the temperature-sensitivity of the virus growth

The test sample and reference Smallpox Vaccine Prepared in Cell Culture (hereafter referred to as "Reference" in this monograph) shall be subjected to serial dilutions and inoculated onto monolayer cultures of rabbit kidney cells. Cultures shall be incubated at  $35 \pm 1^\circ\text{C}$  and  $41.0 \pm 0.5^\circ\text{C}$ , and the ratio of virus yield at  $35 \pm 1^\circ\text{C}$  to that at  $41.0 \pm 0.5^\circ\text{C}$  shall be greater than  $10^5$ .

##### 3.3.2.2 Test for pock morphology

The test and Reference samples shall be subjected to serial dilutions and inoculation onto the chorioallantoic membrane (hereafter referred to as "CAM" in this monograph) of developing chick embryos aged 11 to 12 days. After the incubation at  $35 \pm 1^\circ\text{C}$  for 48 hours, the diameter of pocks formed on CAM shall not be larger than 3mm.

### 3.4 Tests on final product

Following tests shall apply to each final lot. For the products dispensed in glass capillaries, the test 3.4.1 may be substituted by the test 3.3.1

#### 3.4.1 Sterility test

The test given in General Tests and shall apply.

#### 3.4.2 Potency test

The test shall be conducted by measuring pock forming units on CAM of the developing chick embryos.

##### 3.4.2.1 Materials

The test sample, reference Smallpox Vaccine Prepared in Cell Culture (hereafter referred to as "Reference Vaccine" in this monograph), and the Reference shall be used. The diluent shall be 0.0067 mol/L phosphate-buffered sodium chloride solution (pH 7.6) or an equivalent phosphate buffer containing 0.2 w/v% gelatin.

##### 3.4.2.2 Test procedure

The test sample and Reference Vaccine or the Reference shall each be diluted serially into at least three suitable logarithmic levels (hereafter referred to as "test dilutions" and "reference

dilutions" in this monograph).

In at least 10 chick embryos aged 11 to 12 days, incubation shall be performed with each dilution. A dose of 0.1 mL of the dilution shall be inoculated onto the chorioallantoic membrane of each egg, which has been provided with an artificial air sac, and the eggs shall be incubated at  $36 \pm 1^\circ\text{C}$  for 48–72 hours. The dilutions of the test sample and Reference Vaccine or the Reference yielding no less than 10 easily countable pocks per membrane in most membranes shall be chosen for the calculation of pocks.

The mean number of pocks per membrane and its dilution factor shall be used to calculate the number of pock-forming units per mL of the test sample and Reference Vaccine or the Reference. The number for the Reference Vaccine shall be approximately that prescribed on its label.

#### 3.4.2.3 Criterion for judgment

Pock-forming units per mL in the test sample shall be no less than  $10^{7.7}$ .

#### 3.4.3 Identity test

The test shall be carried out by pock formation on chorioallantoic membrane of developing chick embryos and by its neutralization with anti-vaccinia immune serum.

### **4. Storage and expiry date**

#### 4.1 Storage

Storage temperature shall be  $-15^\circ\text{C}$  or less and  $5^\circ\text{C}$  or less before and after delivery from warehouse, respectively.

#### 4.2 Expiry date

The expiry date shall be one year. Expiry date from the delivery from warehouse shall be within three months.

### **5. Other requirements**

#### 5.1 Final product dispensed in glass capillaries

The description of contents on the label for the final product dispensed in glass capillaries shall be, for example, "5 capillaries of 10 doses," where one dose shall be approximately 0.01 mL.

#### 5.2 Labeling

The date of issue

#### 5.3 Information to be provided in package insert and other labeling

- (1) Name of virus strain used for production.
- (2) Names and contents of antibiotics or dyes used in the cultivation of the virus, if applicable.
- (3) Names and contents of the stabilizer, if applicable.
- (4) Route of administration

Multiple pressure method shall apply.

# **FREEZE-DRIED SMALLPOX VACCINE PREPARED IN CELL CULTURE**

## **1. Descriptive definition**

“Freeze-dried Smallpox Vaccine Prepared in Cell Culture” (hereafter referred to as "freeze-dried vaccine" in this monograph) is a freeze-dried product containing live vaccinia virus (hereafter referred to as "virus" in this monograph). When reconstituted, it becomes a yellowish or reddish clear or slightly turbid liquid.

## **2. Production control**

### 2.1 Source materials

#### 2.1.1 Strains of virus

Requirements given in 2.1.1 of Smallpox Vaccine Prepared in Cell Culture shall apply.

#### 2.1.2 Animals used for production

Requirements given in 2.1.2 of Smallpox Vaccine Prepared in Cell Culture shall apply.

#### 2.1.3 Culture media

Requirements given in 2.1.3 of Smallpox Vaccine Prepared in Cell Culture shall apply.

### 2.2 Bulk material

#### 2.2.1 Cell cultures

Requirements given in 2.2.1 of Smallpox Vaccine Prepared in Cell Culture shall apply.

#### 2.2.2 Virus cultivation and harvest

Requirements given in 2.2.2 of Smallpox Vaccine Prepared in Cell Culture shall apply.

### 2.3 Final bulk and freeze-drying

The bulk material may be mixed and, if necessary, diluted to serve as the final bulk.

Appropriate stabilizer may be added. Antibiotics shall not be added.

The final bulk shall be dispensed and freeze-dried. The final bulk shall be subjected to the tests given in 3.3.

## **3. Control tests**

### 3.1 Tests on individual cell cultures

Requirements given in 3.1 of Smallpox Vaccine Prepared in Cell Culture shall apply.

### 3.2 Tests on bulk material

Requirements given in 3.2 of Smallpox Vaccine Prepared in Cell Culture shall apply.

### 3.3 Tests on the final bulk

Requirements given in 3.3 of Smallpox Vaccine Prepared in Cell Culture shall apply.

### 3.4 Tests on the final product

Following tests shall apply to each final lot.

#### 3.4.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### 3.4.2 Sterility test

The test given in General Tests shall apply.

#### 3.4.3 Potency test

Requirements given in 3.4.2 of Smallpox Vaccine Prepared in Cell Culture shall apply.

#### 3.4.4 Stability test

The test given in 3.4.3 shall be applied to the test sample kept at  $37 \pm 1^\circ\text{C}$  for 4 weeks. The number of pock-forming units per mL of the test sample shall be no less than 1/10 the value before warming and shall be no less than  $10^{7.7}$ .

#### 3.4.5 Identity test

Requirements given in 3.4.3 of Smallpox Vaccine Prepared in Cell Culture shall apply.

### **4. Storage and expiry date**

#### 4.1 Storage

Storage temperature shall be  $-20^\circ\text{C}$  or less.

#### 4.2 Expiry date

The expiry date shall be three years.

### **5. Other requirements**

#### 5.1 Reconstituent

The reconstituent shall contain glycerin at an appropriate concentration or other substance to make the solution viscous. The solution shall contain no antibiotic. The volume of the solution shall be 0.01mL for one dose.

#### 5.2. Information to be provided in package insert and other labeling

- (1) Name of the virus strain used for production.
- (2) Names and contents of antibiotics or dyes used in cultivation of the virus, if applicable.
- (3) Names and contents of stabilizer, if applicable.
- (4) Route of administration.

Multiple pressure method shall apply.

# JAPANESE ENCEPHALITIS VACCINE

## 1. Descriptive definition

“Japanese Encephalitis Vaccine” is a colorless clear or slightly whitish turbid liquid product containing inactivated Japanese encephalitis 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

The *Beijing 1* strain or other strain specified elsewhere shall be used.

#### 2.1.2 Animals used for production

Mice aged 3-5 weeks shall be used.

### 2.2 Bulk material

#### 2.2.1 Virus suspension

The brains of mice inoculated intracerebrally with the virus strain for production showing typical signs of encephalitis immediately before death shall be harvested. The harvested brains shall be triturated in buffered physiological saline or other suitable medium, and centrifuged. The supernatant shall be collected, and treated by alcohol precipitation, protamine sulfate, ultracentrifugation or other appropriate method to serve as the virus suspension.

The virus suspension shall be subjected to the tests given in 3.1.

#### 2.2.2 Inactivation of virus

Formalin or other appropriate agents having a comparable effect shall be used for the inactivation of virus. A suspension containing inactivated virus shall serve as the bulk material.

The bulk material shall be subjected to the tests given in 3.2.

### 2.3 Final bulk

Bulk material shall be diluted in buffered physiological saline or other suitable medium to serve as the final bulk. Appropriate preservatives and stabilizer may be added.

## 3. Control tests

### 3.1 Tests on virus suspension

#### 3.1.1 Sterility test

Sterility test and Mycoplasma test given in General Tests shall apply.

### 3.2 Tests on bulk material

#### 3.2.1 Staining test

The test given in General Tests shall apply.

#### 3.2.2 Sterility test

The test given in General Tests shall apply.

### 3.2.3 Inactivation test

A test sample corresponding to no less than 200 mL of the final bulk shall be used. The test sample shall be dialyzed at about 5°C for no less than 24 hours against a sufficient amount of physiological saline, diluted if necessary, to remove any cytopathogenic effect due to residual inactivating agent or other substances, to serve as the dialyzed test material.

The total volume of the dialyzed test material shall be inoculated into a primary culture of hamster kidney cells or other cell culture with no less susceptibility to the virus than the former, and incubated at  $35 \pm 1^\circ\text{C}$  for a period of 14 days. A contiguous cell culture sheet of no less than  $3\text{ cm}^2$  shall be used for 1 mL of the test material.

During the incubation period, no cytopathic change shall be detected.

At the completion of observation, the cultured fluid shall be collected and inoculated intracerebrally at a dose of 0.03 mL into at least 10 mice aged 4 weeks. The animals shall be observed for 14 days.

No animal shall show any abnormal sign during the observation period.

### 3.3 Tests on final product

Following tests shall apply to each final lot.

#### 3.3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

#### 3.3.2 Test for protein content

When the test given in General Tests is applied, the protein content shall be no higher than 80  $\mu\text{g/mL}$ .

#### 3.3.3 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

#### 3.3.4 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%.

#### 3.3.5 Sterility test

The test given in General Tests shall apply.

#### 3.3.6 Inactivation test

The test sample shall be given by intracerebral injection at a dose of 0.03 mL into at least 10 mice aged 4 weeks. The animals shall be observed for 14 days. No animal shall show any abnormal sign during the observation period.

#### 3.3.7 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.3.8 Potency test

Potency shall be determined by titrating the neutralizing antibody produced in the immunized mice by the plaque reduction method using Vero cell cultures.

##### 3.3.8.1 Materials

The test sample, Reference Japanese Encephalitis Vaccine (hereafter referred to as "Reference" in this monograph) and the virus strain for challenge (hereafter referred to as "challenge strain" in this monograph) shall be used.

The diluent for the test sample and Reference shall be the appropriate buffered isotonic solution.

The virus strain for challenge shall be inoculated intracerebrally into suckling mice within 3 days after birth. The brains of the mice showing typical signs of infection shall be harvested and triturated in appropriate diluent to make an emulsion. After centrifugation, the appropriately diluted supernatant shall be used as the challenge virus solution.

#### 3.3.8.2 Test procedures

The test sample and Reference shall be diluted to make appropriate logarithmic serial dilutions. Each dilution shall be given by intraperitoneal injection in 2 doses of 0.5 mL at an interval of 7 days into at least 10 mice aged 4 weeks.

Seven days after the second injection, each animal shall be bled. The same volume of blood shall be taken from each mouse and the serum shall be separated. The serum preparations shall then be heated at 56°C for 30 minutes, and appropriately diluted in Eagle's MEM with FBS. An equal volume of the serum dilution and virus suspension for challenge shall be taken accurately and mixed well. The mixture shall be kept standing at  $36 \pm 1^\circ\text{C}$  for 1.5 hours, and inoculated at a dose of 100  $\mu\text{L}$  into at least 3 wells of Vero cells. The virus suspension for challenge shall be diluted two-fold in Eagle's MEM with FBS. The dilution shall be similarly treated and inoculated at a dose of 100  $\mu\text{L}$  into at least twelve wells of Vero cells to serve as the control. The inoculated cultures shall be kept standing at  $36 \pm 1^\circ\text{C}$  for 1.5 hours in a  $\text{CO}_2$  incubator, overlaid with the overlaying agar medium and incubated for 5–8 days. After culture, all plates shall be inactivated, fixed with formalin and stained with an appropriate pigment.

The number of plaques formed on cultures shall be counted to obtain the reduction rates to the control for the test and Reference groups. The 50% neutralizing antibody titers shall be calculated from these rates for each group.

The mean number of plaques in the control shall be 50–150 per dish.

#### 3.3.8.3 Criterion for judgment

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

#### 3.3.9 Identity test

The test shall be conducted serologically.

### 4. Storage and expiry date

The expiry date shall be one year.

### 5. Other requirements

#### 5.1 Information to be provided in package insert and other labeling

Recommended human dose and route of administration, as follows:

For primary immunization, usually 2 doses of 0.5 mL are given by subcutaneous injection at an

interval of 1–4 weeks.

For booster immunization, usually 0.5 mL is given by subcutaneous injection.

The dose may be reduced to 0.25 mL for those aged below 3 years.

# **FREEZE-DRIED JAPANESE ENCEPHALITIS VACCINE**

## **1. Descriptive definition**

“Freeze-dried Japanese Encephalitis Vaccine” is a freeze-dried product containing inactivated Japanese encephalitis virus (hereafter referred to as “virus” in this monograph). When reconstituted, it becomes a colorless or slightly turbid liquid.

## **2. Production control**

### 2.1 Source materials

#### 2.1.1 Strains of virus used for production

Requirements given in 2.1.1 of Japanese Encephalitis Vaccine shall apply.

#### 2.1.2 Animals used for production

Requirements given in 2.1.2 of Japanese Encephalitis Vaccine shall apply.

### 2.2 Bulk material

#### 2.2.1 Virus suspension

Requirements given in 2.2.1 of Japanese Encephalitis Vaccine shall apply.

#### 2.2.2 Inactivation of virus

Requirements given in 2.2.2 of Japanese Encephalitis Vaccine shall apply.

### 2.3 Final bulk and freeze-drying

The bulk material shall be diluted, if necessary, with buffered physiological saline or other suitable medium, dispensed and freeze-dried. Appropriate preservative and stabilizer may be used.

## **3. Control tests**

### 3.1 Tests on virus suspension

#### 3.1.1 Sterility test

Sterility test and Mycoplasma test given in General Tests shall apply.

### 3.2 Tests on bulk material

#### 3.2.1 Staining test

The test given in General Tests shall apply.

#### 3.2.2 Sterility test

The test given in General Tests shall apply.

#### 3.2.3 Inactivation test

Requirements given in 3.2.3 of Japanese Encephalitis Vaccine shall apply.

### 3.3 Tests on final product

Following tests shall apply to each final lot. If thimerosal is not added, the test given in 3.3.4 shall be omitted.

#### 3.3.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in

General Tests.

3.3.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

3.3.3 Test for protein content

When the test given in General Tests is applied, the protein content shall be no higher than 80 µg/mL.

3.3.4 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

3.3.5 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%.

3.3.6 Sterility test

The test given in General Tests shall apply.

3.3.7 Inactivation test

Requirements given in 3.3.6 of Japanese Encephalitis Vaccine shall apply.

3.3.8 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.3.9 Potency test

Requirements given in 3.3.8 of Japanese Encephalitis Vaccine shall apply.

3.3.10 Identity test

Requirements given in 3.3.9 of Japanese Encephalitis Vaccine shall apply.

**4. Storage and expiry date**

The expiry date shall be five years.

**5. Other requirements**

5.1 Information to be provided in package insert and other labeling

- (1) The vaccine shall be reconstituted with water for injection immediately before injection.
- (2) Statement that no preservatives are added, if applicable.
- (3) Recommended human dose and route of administration, as follows:

Requirements given in 5.1 of Japanese Encephalitis Vaccine shall apply.

# PNEUMOCOCCAL VACCINE POLYVALENT

## 1. Descriptive definition

“Pneumococcal Vaccine Polyvalent” is a colorless clear liquid product containing purified capsular polysaccharides from 23 serotypes of *Streptococcus pneumoniae*. The serotype designations by the Danish naming system are 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.

## 2. Product control

### 2.1 Source materials

#### 2.1.1 Seeds for production

*S. pneumoniae* seeds with serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F shall be used.

#### 2.1.2 Liquid growth medium

The components of *S. pneumoniae* growth medium shall not contain any substances which have the possibility of inducing a pronounced allergic reaction in humans.

### 2.2 Production of polysaccharide bulk powder

#### 2.2.1 Fermentation

Expansion of the *S. pneumoniae* seed of each serotype is performed at  $37 \pm 2^\circ\text{C}$ . The serotypes are confirmed by the Quellung method with type-specific antisera. When the fermentation is finished, the culture shall not contain any contaminants. The testing methods are microscopic examination by Gram staining, culture with a blood agar plate and serotyping.

#### 2.2.2 Inactivation

*S. pneumoniae* culture in the production fermentor is inactivated by the addition of liquid phenol. The inactivation process is performed at  $22\text{--}37^\circ\text{C}$  for 2 hours in the presence of 1w/v% phenol.

#### 2.2.3 Purification for producing the bulk powder

Purification of each of the 23 *pneumococcal* capsular polysaccharides from inactivated *S. pneumoniae* fermentation broth is accomplished by centrifugation to remove cell debris and nucleic acids after the addition of ethanol. The *pneumococcal* polysaccharides are recovered by the alcohol fractionation to obtain crude polysaccharides. The polysaccharide powder is recovered by subsequent purification processes including proteolytic enzyme digestion, and salting out and/or sedimentation by organic solvents. The powder is then vacuum-dried at  $20\text{--}25^\circ\text{C}$  to obtain the bulk powder. The bulk powder is stored at  $-20^\circ\text{C}$  or less. Each bulk powder shall be subjected to the test given in 3.1.

### 2.3 Production of the final (polyvalent) bulk

Individual aliquots of each of the 23 polysaccharide powders are dissolved in buffered physiological saline and mixed to produce the polyvalent bulk. The polyvalent bulk contains each polysaccharide at the concentration of  $50 \mu\text{g/mL}$ . After adding phenol at the final concentration of 0.25 w/v%, the polyvalent bulk shall be passed through  $0.20 \mu\text{m}$  filter to obtain the sterile final (polyvalent) bulk.

### 3. Control tests

#### 3.1 Tests on the bulk powders

##### 3.1.1 O-Acetate content

On appropriate assay, O-acetate content of the following serotype shall be equal to or greater than the following specification.

| Serotype | O-Acetate (%) |
|----------|---------------|
| 1        | 1.8           |

##### 3.1.2 Uronic acid content

On appropriate assay, uronic acid content of each serotype shall be equal to or greater than the following specifications.

| Serotype | Uronate (%) |
|----------|-------------|
| 1        | 45.0        |
| 2        | 15.0        |
| 3        | 40.0        |
| 5        | 12.0        |
| 8        | 25.0        |
| 9N       | 20.0        |
| 9V       | 15.0        |
| 22F      | 15.0        |

##### 3.1.3 Methypentose content

On appropriate assay, methylpentose content of each serotype shall be equal to or greater than the following specifications.

| Serotype | Methylpentose (%) |
|----------|-------------------|
| 2        | 38.0              |
| 6B       | 15.0              |
| 7F       | 13.0              |
| 17F      | 25.0              |
| 18C      | 14.0              |
| 19A      | 15.0              |
| 19F      | 20.0              |
| 22F      | 25.0              |
| 23F      | 37.0              |

#### 3.1.4 Hexosamine content

On appropriate assay, hexosamine content of each serotype shall be equal to or greater than the following specifications.

| Serotype | Hexosamine (%) |
|----------|----------------|
| 4        | 40.0           |
| 5        | 25.0           |
| 9N       | 28.0           |
| 9V       | 13.0           |
| 10A      | 12.0           |
| 12F      | 25.0           |
| 14       | 20.0           |
| 15B      | 15.0           |
| 19A      | 15.0           |
| 19F      | 12.5           |
| 20       | 15.0           |

#### 3.1.5 Protein content

On appropriate assay, the protein content of each serotype shall be equal to or less than the following specifications.

| Serotype | Protein content (%) |
|----------|---------------------|
| 1        | 2.0                 |
| 2        | 2.0                 |
| 3        | 5.0                 |
| 4        | 3.0                 |
| 5        | 6.5                 |
| 6B       | 2.0                 |
| 7F       | 5.0                 |
| 8        | 2.0                 |
| 9N       | 2.0                 |
| 9V       | 2.0                 |
| 10A      | 3.0                 |
| 11A      | 2.0                 |
| 12F      | 3.0                 |

|     |     |
|-----|-----|
| 14  | 5.0 |
| 15B | 2.0 |
| 17F | 2.0 |
| 18C | 3.0 |
| 19A | 2.0 |
| 19F | 3.0 |
| 20  | 2.0 |
| 22F | 3.0 |
| 23F | 2.0 |
| 33F | 2.0 |

### 3.1.6 Nucleic acid content

On absorbance assay as specified in JP, the nucleic acid content of each serotype shall be equal to or less than the following specifications.

| Serotype | Nucleic acid (%) |
|----------|------------------|
| 1        | 2.0              |
| 2        | 2.0              |
| 3        | 2.0              |
| 4        | 2.0              |
| 5        | 2.0              |
| 6B       | 2.0              |
| 7F       | 2.0              |
| 8        | 2.0              |
| 9N       | 1.0              |
| 9V       | 2.0              |
| 10A      | 2.0              |
| 11A      | 2.0              |
| 12F      | 2.0              |
| 14       | 2.0              |
| 15B      | 2.0              |
| 17F      | 2.0              |
| 18C      | 2.0              |

|     |     |
|-----|-----|
| 19A | 2.0 |
| 19F | 2.0 |
| 20  | 2.0 |
| 22F | 2.0 |
| 23F | 2.0 |
| 33F | 2.0 |

### 3.1.7 Nitrogen contents

On nitrogen quantitative assay as specified in JP, or in the General Tests, the nitrogen content of each serotype shall be equal to the following specifications.

| Serotype | Nitrogen content (%) |
|----------|----------------------|
| 1        | 3.5–6.0              |
| 2        | ≤1.0                 |
| 3        | ≤1.0                 |
| 4        | 4.0–6.0              |
| 5        | 2.5–5.5              |
| 6B       | ≤2.0                 |
| 7F       | 2.0–4.0              |
| 8        | ≤1.0                 |
| 9N       | 2.2–4.0              |
| 9V       | 1.0–3.0              |
| 10A      | 0.5–3.0              |
| 11A      | ≤2.0                 |
| 12F      | 3.0–5.0              |
| 14       | 1.5–4.0              |
| 15B      | 1.0–3.0              |
| 17F      | ≤1.0                 |
| 18C      | ≤1.0                 |
| 19A      | 1.5–3.5              |
| 19F      | 1.4–3.5              |
| 20       | 1.0–2.0              |
| 22F      | ≤1.0                 |

|     |      |
|-----|------|
| 23F | ≤1.0 |
| 33F | ≤2.0 |

### 3.1.8 Phosphorus content

On appropriate assay, the phosphorus contents of each serotype shall be equal to the following specifications.

| Serotypes | Phosphorus (%) |
|-----------|----------------|
| 1         | ≤1.5           |
| 2         | ≤1.0           |
| 3         | ≤1.0           |
| 4         | ≤1.5           |
| 5         | ≤2.0           |
| 6B        | 3.0–4.5        |
| 7F        | ≤1.0           |
| 8         | ≤1.0           |
| 9N        | ≤1.0           |
| 9V        | ≤1.0           |
| 10A       | 1.5–3.5        |
| 11A       | 2.0–5.0        |
| 12F       | ≤1.0           |
| 14        | ≤1.0           |
| 15B       | 2.0–5.0        |
| 17F       | 1.5–3.5        |
| 18C       | 2.4–4.9        |
| 19A       | 3.0–7.0        |
| 19F       | 3.0–5.5        |
| 20        | 2.0–4.0        |
| 22F       | ≤1.0           |
| 23F       | 3.0–4.5        |
| 33F       | ≤1.0           |

### 3.1.9 Average molecular mass

On gel filtration assay using the specified lot of Sepharose 4B and 0.2 mol/L ammonium acetate or on equivalent assay, the K<sub>d</sub> value of each serotype shall be equal to or less than the following

specifications.

| Serotypes | Kd value |
|-----------|----------|
| 1         | 0.15     |
| 2         | 0.15     |
| 3         | 0.15     |
| 4         | 0.15     |
| 5         | 0.30     |
| 6B        | 0.20     |
| 7F        | 0.20     |
| 8         | 0.15     |
| 9N        | 0.20     |
| 9V        | 0.20     |
| 10A       | 0.20     |
| 11A       | 0.20     |
| 12F       | 0.25     |
| 14        | 0.30     |
| 15B       | 0.20     |
| 17F       | 0.20     |
| 18C       | 0.15     |
| 19A       | 0.40     |
| 19F       | 0.20     |
| 20        | 0.25     |
| 22F       | 0.20     |
| 23F       | 0.15     |
| 33F       | 0.25     |

#### 3.1.10 Serological identity test

The bulk powder of each serotype shall be tested by in-gel precipitation (Ouchterlony) assay using type-specific rabbit antisera.

#### 3.1.11 A, B Blood group substance testing

##### 3.1.11.1 Materials

The polysaccharide powder is dissolved in water (1400 µg/mL). Anti-A and anti-B sera, blood group A and B erythrocytes and the 0.05% blood group A- and B-specific Standard Antigens (50 µg/0.1 mL, sourced from the stock culture collection of a governmental institute) are used. The

antisera are serially diluted at a ratio of 2 with saline. The erythrocytes are suspended in physiological saline at 1 vol%. The suspensions in an amount of 0.1 mL each are mixed with the same volume of the diluted antisera, and incubated at room temperature for 30 minutes. The erythrocytes are centrifuged at about  $190 \times g$  for 1 minute. One unit of the agglutination titer is defined as the maximal dilution of sera agglutinating the erythrocytes. Two units of antisera are used for testing. The blood group A- and B-specific antigens are used to determine sensitivity and are diluted from 1:8 to 1:4096 by serial double dilution.

#### 3.1.11.2 Test

A 0.1-mL portion of the sample, a 0.1-mL portion of physiological saline (for antisera control), a 0.2-mL portion of physiological saline (for erythrocyte control) and the serially diluted blood group A- or B-specific antigens are prepared for each blood type. A 0.1-mL portion of antisera corresponding to 2 units is added to all tubes, except tubes containing the erythrocyte control. After gentle shaking and incubation at room temperature for 10 minutes, 0.1 mL of the corresponding erythrocyte suspensions are added. After gentle shaking, incubation at room temperature for 30 minutes and centrifugation at about  $190 \times g$  for 1 minute, agglutination is checked by the unaided eye. The sensitivity of the test is calculated by dividing  $50 \mu\text{g}/0.1 \text{ mL}$  (the concentration of the released Standard Antigens) by the maximum dilution at which the blood group A- or B-specific antigens can inhibit the agglutination.

#### 3.1.11.3 Criterion for judgment

The test results meet the following requirements.

- (1) The detection sensitivity of group A antigens is equal to or less than  $0.4 \mu\text{g}/0.1 \text{ mL}$ .
- (2) The detection sensitivity of group B antigens is equal to or less than  $0.4 \mu\text{g}/0.1 \text{ mL}$ .
- (3) Agglutination is observed in the antisera control.
- (4) Agglutination is not observed in the erythrocyte control.
- (5) Agglutination is observed in the sample.

### 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, pH of the test sample shall be within the range of 6.0 to 7.5.

#### 3.2.2 Test for phenol concentration

When the test given in General Tests is applied, the phenol concentration shall be within the range of 0.225% to 0.275%.

The test sample shall be the 25-fold diluted final product.

#### 3.2.3 Sterility test

The test given in General Tests shall apply.

#### 3.2.4 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.2.5 A, B Blood group substance testing

##### 3.2.5.1 Materials

Anti-A and anti-B sera and blood group A and B erythrocytes are used. The antisera are diluted by two-fold serial dilution with saline. The erythrocytes are suspended in saline at 1%. A 0.1-mL portion of the suspensions is well mixed with the same volume of the diluted antisera and incubated at room temperature for 30 minutes. The erythrocytes are centrifuged at about  $190 \times g$  for 1 minute. One unit of the agglutination titer is defined as the maximal dilution of sera agglutinating the erythrocytes. Two units of the antisera are used for the testing.

#### 3.2.5.2 Test

A 0.1-mL portion of the sample, a 0.1-mL portion of saline (for antisera control) and a 0.2-mL portion of physiological saline (for erythrocyte control) are prepared for each blood type. A 0.1-mL portion of the antisera corresponding to 2 units is added to all tubes, except tubes containing the erythrocyte control. After gentle shaking and incubation at room temperature for 10 minutes, 0.1 mL of the corresponding erythrocyte suspensions are added. After gentle shaking, incubation at room temperature for 30 minutes and centrifugation at about  $190 \times g$  for 1 minute, agglutination is checked macroscopically.

#### 3.2.5.3 Criterion for judgment

The test results shall meet the following requirements.

- (1) Agglutination is observed in the antisera control.
- (2) Agglutination is not observed in the erythrocyte control.
- (3) Agglutination is observed in the sample.

#### 3.2.6 Identity test

The test given in 3.1.10 shall apply. If necessary, the sample can be appropriately diluted with physiological saline containing 0.25 w/v% phenol. The sample reacts with the 23 type-specific antisera to produce precipitates.

### **4. Storage and expiry date**

The expiry date shall be two years.

### **5. Other requirements**

5.1 Information to be provided in package insert and other labeling.

- (1) Re-administration shall be avoided irrespective of the period from the last administration.
- (2) Recommended human dose and route of administration, as follows:

Generally, one dose of 0.5 mL is given by intramuscular or subcutaneous injection.

# TETANUS TOXOID

## 1. Descriptive definition

“Tetanus Toxoid” is a colorless or slightly yellowish brown clear liquid product containing "tetanus toxoid" (hereafter referred to as "toxoid" in this monograph), which is prepared by treating tetanus toxin (hereafter referred to as "toxin" in this monograph) with formaldehyde (hereafter referred to as "toxoiding" in this monograph) by a method minimally impairing immunogenicity.

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Strains of *Clostridium tetani*

*Clostridium tetani* Harvard strain or other strains with equivalent or higher toxigenicity shall be used.

#### 2.1.2 Culture medium

The medium used for production of toxin shall be free from substances of horse or human origin, specific human blood group substances, or other substances possibly inducing severe allergic reactions in human.

### 2.2 Bulk material

#### 2.2.1 Toxic filtrate

Cultures of *C. tetani* after incubation shall be tested for bacterial purity by microscopic examination or inoculation into appropriate culture media. Cultures shown to be free from any contaminating microorganisms shall be filtered through a filter capable of yielding bacteriologically sterile filtrate.

The 1 L<sub>+</sub> dose of the toxic filtrate shall be no more than 0.05 mL, or the filtrate shall contain at least 20 Lf/mL when tested by the method given in 3.3.8.

#### 2.2.2 Toxoiding and purification

Formaldehyde shall be used for toxoiding. Purification shall be performed either before or after toxoiding. The preparation containing purified toxoid shall serve as the bulk material.

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

### 2.3 Final bulk

The bulk material shall be diluted in buffered physiological saline or other suitable medium to a concentration not higher than 50 Lf of toxoid per mL to serve as the final bulk. Appropriate stabilizer may be added.

## 3. Control tests

### 3.1 Tests on bulk material

#### 3.1.1 Purity test

When the protein nitrogen content and toxoid content are determined by the test given in General Tests and the test given in 3.2.8, respectively, the bulk material shall contain no less than 1,500 Lf toxoid per mg protein nitrogen.

#### 3.1.2 Sterility test

The test given in General Tests shall apply.

#### 3.1.3 Detoxification test

The test given in 3.2.6 shall apply to the following two kinds of samples. One shall be prepared

by diluting the test sample with 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) to contain 100 Lf of toxoid per mL. The other sample contains toxoid of the equivalent or higher concentration as the final bulk, which is no higher than 50 Lf of toxoid per mL. The latter sample shall be kept at 37°C for 20 days prior to the test.

### 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 6.6 to 7.4.

#### 3.2.2 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

#### 3.2.3 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%.

#### 3.2.4 Sterility test

The sterility test given in General Tests shall apply.

#### 3.2.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.2.6 Detoxification test

The test shall be conducted on the test sample as well as on a test sample kept at 37°C for 20 days. Each sample shall be given by subcutaneous injection at a dose of 5 mL into at least 4 guinea pigs weighing 300–400 g. The inoculated animals shall be observed for at least 21 days. No animal shall die due to intoxication, or show specific symptoms of intoxication such as spasms, stiffening, pronounced decrease in body weight, or other abnormal signs during the observation period.

#### 3.2.7 Potency test

Potency shall be measured in guinea pigs or mice by the tetanus toxin challenge method or by the antitoxin titration method.

##### 3.2.7.1 Toxin challenge method

###### 3.2.7.1.1 Materials

The test sample, Standard Tetanus Toxoid (hereafter referred to as "Standard" in this monograph) and an appropriate solution of toxin shall be used. The test sample and the Standard shall be diluted in 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.02 w/v% gelatin, and the toxin in 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

###### 3.2.7.1.2 Test procedures

The test sample and the Standard shall be diluted serially at equal logarithmic intervals. Each dilution shall be given by subcutaneous injection into at least 10 guinea pigs weighing 300–400 g at a dose of 2 mL or at least 10 mice aged 4 weeks at a dose of 0.5 mL. The guinea pigs or mice shall be challenged with approximately 50 or 100 LD<sub>50</sub> of toxin, respectively, 4–6 weeks after immunizing injection. The challenged animals shall be observed for 4 days.

The toxin used for challenge shall be titrated by injection of at least three serial dilutions into at least 3 guinea pigs each weighing 400–600 g or at least 3 mice of equivalent age as the immunized mice. The challenge toxin shall contain 25 to 100 LD<sub>50</sub> per inoculum for guinea pigs or 50 to 200 LD<sub>50</sub> per inoculum for mice.

###### 3.2.7.1.3 Criterion for judgment

The potency of the test sample shall be no less than 30 IU/mL upon statistical analysis.

#### 3.2.7.2 Antitoxin titration method

##### 3.2.7.2.1 Materials

The test sample, the Standard and a toxin solution with a known binding capacity shall be used. Dilutions of these materials shall be made according to 3.2.7.1.1.

##### 3.2.7.2.2 Test procedures

Immunization of animals shall be conducted by the method given in 3.2.7.1.2. Serum shall be taken from each animal 4 to 6 weeks after immunization. When the antitoxin content is determined by the mouse method, the Test for Tetanus Antitoxin Titer Determination given in General Tests shall apply. Standard Tetanus Antitoxin shall be used for the Standard sample.

##### 3.2.7.2.3 Criterion for judgment

The criterion provided in 3.2.7.1.3 shall apply.

#### 3.3.8 Identity test

The test shall be conducted by the flocculation test.

#### **4. Storage and expiry date**

The expiry date shall be two years.

#### **5. Other requirements**

##### 5.1 Information to be provided in package insert and other labeling

For primary immunization, generally 3 doses of 0.5 mL are given by subcutaneous injection at an interval of 3–8 weeks.

For the first booster immunization, generally 0.5 mL is given by subcutaneous injection with the interval of not shorter than 6 months after the completion of the primary immunization (in general, 12–18 months after the completion of the primary immunization).

To those who showed severe untoward reactions at the primary immunization, the dose shall be adequately reduced. This precaution shall be valid for the subsequent booster immunization.

## ADSORBED TETANUS TOXOID

### 1. Descriptive definition

“Adsorbed Tetanus Toxoid” is a liquid product containing "tetanus toxoid" (hereafter referred to as "toxoid" in this monograph), which is prepared by treating tetanus toxin with formaldehyde by a method minimally impairing the immunogenicity of the toxin and rendered the toxin insoluble by adding aluminium salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

### 2. Production control

#### 2.1 Source materials

Requirements given in 2.1 of Tetanus Toxoid shall apply.

#### 2.2 Bulk material

Requirements given in 2.2 of Tetanus Toxoid shall apply.

#### 2.3 Final bulk

The bulk material shall be diluted with buffered physiological saline or other suitable medium and added with an aluminium salt. The final bulk shall contain toxoid of no higher than 20 Lf as protein per mL. Appropriate stabilizer may be added.

### 3. Control tests

#### 3.1 Tests on bulk material

The test given in 3.1 of Tetanus Toxoid 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 5.4 to 7.4.

##### 3.2.2 Test for aluminium content

When the test given in General Tests is applied, the aluminium content shall be no higher than 0.5mg/mL.

##### 3.2.3 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

##### 3.2.4 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%.

##### 3.2.5 Sterility test

The test given in General Tests shall apply.

##### 3.2.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

##### 3.2.7 Detoxification test

The test given in 3.2.6 of Tetanus Toxoid shall apply.

##### 3.2.8 Potency test

Requirements given in 3.2.7 of Tetanus Toxoid shall apply; however, the Standard Tetanus Toxoid in 3.2.7.1.1 shall read Standard Adsorbed Tetanus Toxoid. The diluent for the test sample and Standard shall be physiological saline. When article 3.2.7.1.3 of Standard Tetanus Toxoid is referred

#### **4. Storage and expiry date**

The expiry date shall be two years.

#### **5. Other requirements**

5.1 Information to be provided in package insert and other labeling

- (1) Caution that the product be rendered homogenous by thorough shaking before use
- (2) Recommended human dose and route of administration, as follows:

For primary immunization, generally 2 doses of 0.5 mL are given by intramuscular or subcutaneous injection at an interval of 3–8 weeks.

For the first booster immunization, generally 0.5 mL is given by intramuscular or subcutaneous injection with the interval of not shorter than 6 months after the completion of the primary immunization (in general, 12–18 months after the completion of the primary immunization).

To those who showed severe untoward reactions at the primary immunization, the dose shall be adequately reduced. This precaution shall be valid for the subsequent booster immunization.

## **FREEZE-DRIED HABU ANTIVENOM, EQUINE**

### **1. Descriptive definition**

"Freeze-dried Habu Antivenom" is a dried product containing "Habu (*Trimeresurus flavoviridis*) antivenom" in immunoglobulin of horses (hereafter referred to as "antivenom" in this monograph). When reconstituted, it becomes a colorless or slightly yellowish brown, clear or slightly whitish turbid liquid.

### **2. Production control**

#### 2.1 Source materials

##### 2.1.1 Antigens used for immunization

The venom of Habu (*Trimeresurus flavoviridis*) or a toxoid derived from it shall be used for immunization.

##### 2.1.2 Animals used for production

Horses shall be used for production.

#### 2.2 Bulk material

##### 2.2.1 Crude antivenom material

Crude serum or plasma shall be used if it contains no less than 100 units of antivenom per mL with respect to the anti-lethal toxin, anti-HR1 and anti-HR2, and passes the sterility test and the pyrogen test given in General Tests.

##### 2.2.2 Purification

The fractions containing immunoglobulin shall be prepared by fractionating the crude antivenom material using a suitable method that has been shown not to cause the deterioration of antibodies. The fractions shall be treated with an appropriate proteolytic enzyme. The preparation containing the treated antitoxin shall serve as the bulk material.

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

#### 2.3 Final bulk and freeze drying

The bulk material shall be diluted, if necessary, with buffered physiological saline or other suitable medium to contain no lower than 300 units of antivenom with respect to each of the anti-lethal toxins, anti-HR1 and anti-HR2. The final bulk shall be dispensed into final containers and freeze-dried.

### **3. Control test**

#### 3.1 Tests on bulk material

##### 3.1.1 Test for immunoglobulin content

When the Cellulose Acetate Membrane Electrophoretic Test given in General Tests is applied, no less than 95% of the total proteins shall be immunoglobulin.

##### 3.1.2 Test for freedom from residual proteolytic enzyme

When measured by a suitable method for the detection of proteolytic enzyme activity, the test material shall be practically free from residual proteolytic enzyme activity.

##### 3.1.3 Sterility test

The sterility test given in General Tests shall apply.

#### 3.1.4 Pyrogen test

The test given in General Tests shall apply.

#### 3.1.5 Test for antivenom content

The test given in 3.2.7 shall apply.

### 3.2 Tests on final product

Following tests shall apply to each final lot.

#### 3.2.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### 3.2.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

#### 3.2.3 Test for protein content

When the test for protein nitrogen content provided in General Tests is applied, the total protein content shall be no higher than 40 mg per 300 units with regard to the lowest antivenom titer among the anti-lethal toxins, anti-HR1 and anti-HR2.

#### 3.2.4 Sterility test

The test given in General Tests shall apply.

#### 3.2.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.2.6 Pyrogen test

The test given in General Tests shall apply.

#### 3.2.7 Potency test

Potency shall be determined with respect to the anti-lethal toxins, anti-HR1 and anti-HR2.

##### 3.2.7.1 Determination of anti-lethal toxin titer

###### 3.2.7.1.1 Materials

The test sample, Standard Habu Antivenom (hereafter referred to as "Standard" in this monograph) and Habu Test Venom (Toxin I) shall be used. Dilution of these materials shall be made with 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

###### 3.2.7.1.2 Test procedures

The Standard shall be diluted to five levels at appropriate intervals determined with careful consideration to the accuracy of the test, containing 10.0 units per 0.1 mL for the median dilution (hereafter referred to as "standard dilution" in this monograph). Similarly, a series of five dilutions shall be made with the test sample (hereafter referred to as "test dilution" in this monograph). Habu Test Venom (Toxin I) shall be so diluted as to contain one test dose per 0.1 mL (hereafter referred to as "venom dilution" in this monograph).

A volume shall be taken accurately from each of the Standard and test dilutions, combined with an equal volume of venom dilution and mixed well. Each mixture shall be kept standing for 1 hour

and then injected intravenously at a dose of 0.2 mL into at least 4 mice aged 23-29 days. The animals shall be observed for 2 days after injection.

#### 3.2.7.1.3 Criterion for judgment

The titer for anti-lethal toxin of the test sample shall be determined by statistical analysis of test results.

The final product shall contain antivenom of no less than the value stated on the label.

#### 3.2.7.2 Determination of anti-HR1 titer

##### 3.2.7.2.1 Materials

The test sample, the Standard and the Habu Test Venom (HR1) shall be used. Dilution of these materials shall be made with 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v % gelatin.

##### 3.2.7.2.2 Test procedures

The Standard shall be diluted to five levels at appropriate intervals determined with careful consideration to the accuracy of the test, containing 1.0 unit per 0.1 mL for the median dilution (hereafter referred to as "standard dilution" in this monograph). Similarly, a series of five dilutions shall be made with the test sample (hereafter referred to as "test dilution" in this monograph).

Habu Test Venom (HR1) shall be so diluted as to contain one test dose per 0.1 mL (hereafter referred to as "venom dilution" in this monograph).

A volume shall be taken accurately from each of the Standard and test dilutions, combined with an equal volume of venom dilution and mixed well. Each mixture shall be kept standing for 1 hour and then injected intracutaneously at a dose of 0.2 mL into rabbits weighing 2.0–3.0 kg. At least 2 injections at different sites shall be made with each mixture. Animals shall be killed by anesthetic overdose approximately 24 hours after injection and the skin stripped off. The cross-diameter of hemorrhagic spots shall be measured from the inner surface of the skin.

##### 3.2.7.2.3 Criterion for judgment

An anti-HR1 titer of the test sample shall be determined by statistical analysis of the size of the hemorrhagic spots.

The final product shall contain antivenom of no less than the value stated on the label.

#### 3.2.7.3 Determination of anti-HR2 titer

The test given in 3.2.7.2 shall apply; however, Habu Test Venom (HR1) and anti-HR1 titer shall read Habu Test Venom (HR2) and anti-HR2 titer, respectively.

#### 3.2.8 Identity test

The test shall be conducted by a suitable method.

### **4. Storage and expiry date**

The expiry date shall be 10 years.

### **5. Other requirements**

#### 5.1 Antivenom content of final containers

A sealed final container shall contain antivenom of no less than 6,000 units with respect to each

of the anti-lethal toxins, anti-HR1 and anti-HR2.

## 5.2 Description on the label

Antivenom content in units per mL with respect to each antivenom.

## ADSORBED HABU-VENOM TOXOID

### 1. Descriptive definition

“Adsorbed Habu-venom Toxoid” is a liquid product containing "Habu-venom toxoid" (hereafter referred to as "toxoid" in this monograph), which is prepared by treating toxic substance(s) produced by *Trimeresurus flavoviridis* (hereafter referred to as "Habu venom" in this monograph) with formaldehyde by a method minimally impairing immunogenicity (hereafter referred to as "toxoiding" in this monograph) and rendered insoluble by adding aluminium salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

### 2. Production control

#### 2.1 Source materials

Dried Habu venom shall be used.

#### 2.2 Bulk material

##### 2.2.1 Venom solution

Dried Habu venom shall be dissolved in buffered physiological saline or other suitable medium and filtered through a filter capable of yielding bacteriologically sterile filtrate.

##### 2.2.2 Toxoiding and purification

Formaldehyde shall be used for toxoiding. Purification shall be conducted either before or after toxoiding. The preparation containing purified toxoid shall serve as the bulk material.

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

#### 2.3 Final bulk

The bulk material shall be diluted with buffered physiological saline or other suitable medium and supplemented with aluminium salt. The final bulk shall contain toxoid of no higher than 1 mg protein per mL. Appropriate preservatives and stabilizer may be added.

### 3. Control tests

#### 3.1 Tests on bulk material

##### 3.1.1 Purity test

When the bulk material is diluted to a concentration equivalent to that of the final bulk and tested for protein nitrogen content by the test given in General Tests, the protein content shall be no higher than 1.0 mg/mL.

##### 3.1.2 Sterility test

The sterility test given in General Tests shall apply.

##### 3.1.3 Detoxification test

The following tests shall be conducted on 2 test samples. One shall be prepared by diluting the test sample with 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) to contain toxoid 3 times that in the final bulk in each mL. The other sample shall be diluted to a concentration equivalent to that of the final bulk and kept at 37°C for 20 days prior to the test.

#### 3.1.3.1 Tests in rabbits

The test given in 3.2.7.1 shall apply.

#### 3.1.3.2 Tests in mice

The sample shall be given by intravenous injection at a dose of 0.2 mL into at least 4 mice aged 23–29 days. No animal shall show symptoms specific to intoxication due to Habu venom or other abnormal signs during the 2-day observation period.

### 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 5.4 to 7.4.

#### 3.2.2 Test for aluminium content

When the test given in General Tests is applied, the aluminium content shall be no higher than 1.0 mg/mL.

#### 3.2.3 Test for thimerosal content

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

#### 3.2.4 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%.

#### 3.2.5 Sterility test

The sterility test given in General Tests shall apply.

#### 3.2.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.2.7 Detoxification test

The test shall be conducted on the test sample and that kept at 37°C for 20 days.

##### 3.2.7.1 Test in rabbits

The test shall be conducted with each of the samples and a dilution containing 10 minimum hemorrhagic doses per mL of Habu venom of known hemorrhagic activity in 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin. At least 2 rabbits weighing 2.0–3.0 kg shall be given by intracutaneous injection with each of the samples and the dilution of Habu venom at a dose of 0.2 mL. The rabbits shall be observed for 24 hours.

None of the sites injected with the samples shall show specific response, whereas the site injected with Habu venom dilution shall show specific response during the observation period.

##### 3.2.7.2 Test in mice

Each of the samples shall be given by intraperitoneal injection into at least 4 mice aged 23–29 days at a dose of 0.5 mL. The mice shall be observed for 2 days.

None of the animals shall show specific symptoms of intoxication due to Habu venom or other abnormal signs during the observation period.

#### 3.2.8 Potency test

Potency shall be determined in guinea pigs by antivenom titration methods.

#### 3.2.8.1 Materials

The test sample, Reference Habu-venom Toxoid (hereafter referred to as "Reference"), Standard Habu Antivenom, Habu Test Venom (HR1) and Habu Test Venom (HR2) shall be used. The Standard Habu Antivenom and Habu Test Venoms shall be diluted in 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

#### 3.2.8.2 Test procedures

Each of the logarithmic serial doses of the test sample and the Reference shall be given by subcutaneous injection into at least 10 guinea pigs weighing 300–400 g. The injection shall be done twice at a 4–5 week interval. Each animal shall be bled 10–20 days after the second injection. The Antivenom content shall be determined by the method given in 3.2.7 of Habu Antivenom with respect to anti-HR1 and anti-HR2.

#### 3.2.8.3 Criterion for judgment

The potency of the test sample shall be no less than 5 units per mL with respect to anti-HR1 and no less than 1 unit/mL with respect to anti-HR2 by statistical analysis of test results.

#### 3.2.9 Identity test

The test shall be conducted by the precipitation reaction using Habu antivenom on the test sample solubilized with sodium citrate or other suitable reagents.

### **4. Storage and expiry date**

The expiry date shall be three years.

### **5. Other requirements**

#### 5.1 Information to be provided in package insert and other labeling

- (1) Caution that the product be rendered homogenous by thorough shaking before use
- (2) Recommended human dose and route of administration, as follows:

For primary immunization, generally 2 doses of 0.5 mL are given by intramuscular subcutaneous injection at an interval of 2–4 weeks.

For booster immunization, generally 0.5 mL is given by intramuscular or subcutaneous injection 4–6 months after the primary immunization.

To those who showed severe untoward reactions at the primary immunization, the dose shall be adequately reduced. This precaution shall be valid for the subsequent booster immunization.

# ADSORBED HEPATITIS B VACCINE

## 1. Descriptive definition

“Adsorbed Hepatitis B Vaccine” is a liquid product containing "hepatitis B surface antigen" (hereafter referred to as 'HBsAg' in this monograph) rendered insoluble by the addition of aluminum salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

## 2. Production control

### 2.1 Source material

Source plasma shall be collected according to the requirements given in 4.1 of Materials from Organisms. Among these, materials positive for HBsAg shall be used. The test for nucleic acid amplification (hereafter referred to as NAT in this monograph) against, at the minimum, RNAs of hepatitis C virus (hereafter referred to as HCV in this monograph) and human immunodeficiency virus (hereafter referred to as HIV in this monograph) shall be applied against source plasma, except when the blood materials were already confirmed negative by appropriate NAT. Plasma in which RNAs of either HCV or HIV are detected shall not be used as source plasma.

### 2.2 Bulk material

#### 2.2.1 Source plasma

Source plasma shall be separated from blood by a suitable method at 4–10°C. Frozen plasma positive for HBsAg may be used after thawing, if it fulfills all other requirements of 'Fresh-Frozen Human Plasma'. The separated plasma shall be pooled to serve as the source plasma. The source plasma shall be subjected to the tests given in 3.1. When the source plasma is to be stored, it shall be kept at 5°C or less.

#### 2.2.2 Purified HBsAg suspension

HBsAg shall be concentrated and purified from the source plasma using suitable procedures. The purified HBsAg suspension shall be subjected to the tests given in 3.2.

#### 2.2.3 Inactivation

Hepatitis B virus shall be inactivated by heating and the addition of formalin. Heating shall be conducted at  $60.0 \pm 0.5^\circ\text{C}$  for more than 10 hours. Treatment with formalin shall be conducted at  $37^\circ\text{C}$  for 96 hours in the presence of at least 0.018 w/v% formaldehyde. The purified HBsAg suspension resulting from the inactivation shall serve as the bulk material. The bulk material shall be subjected to the tests given in 3.3.

### 2.3 Final bulk

The bulk material shall be diluted in buffered physiological saline or other suitable medium, and supplemented with aluminum salt to serve as the final bulk. Appropriate preservative may be used.

## 3. Control tests

### 3.1 Tests on source plasma

#### 3.1.1 Sterility tests

Sterility test, Mycoplasma test, and Mycobacterium sterility test given in General Tests shall apply.

### 3.1.2 Tests for freedom from extraneous viruses

#### 3.1.2.1 Tests in animals

##### 3.1.2.1.1 Inoculation into adult mice

At least 10 adult mice aged 4 to 5 weeks, shall be inoculated intraperitoneally with 0.5mL and intracerebrally with 0.03 mL of the test sample. The mice shall be observed for 21 days. No animal shall show evidence of infection with extraneous microbiological agents, and more than 80% of the animals shall survive the observation period.

##### 3.1.2.1.2 Inoculation into suckling mice

At least 20 suckling mice, shall be inoculated intraperitoneally with 0.1 mL and intracerebrally with 0.01mL of the test sample within 24 hours of birth. The mice shall be observed for 14 days. No animal shall show evidence of infection with extraneous microbiological agents, and more than 80% of the animals shall survive the observation period.

#### 3.1.2.2 Tests in cell culture

##### 3.1.2.2.1 Inoculation of human cell culture

A 5-mL portion of the test sample shall be inoculated in a human cell culture. The culture shall be observed for 14 days. No cytopathic change due to extraneous viruses shall be detected. The test shall be conducted either in WI-38 cells or in MRC-5 cells.

##### 3.1.2.2.2 Inoculation of monkey cell culture

A 5-mL portion of the test sample shall be inoculated in a monkey cell culture. The culture shall be observed for 14 days. No cytopathic change due to extraneous viruses shall be detected. The test shall be conducted either in *Cercopithecus* monkey kidney primary cell cultures or in VERO cells.

#### 3.1.2.3 Tests in embryonated chicken egg

At least 20 embryonated chicken eggs aged 10–11 days shall be inoculated by the allantoic route with 0.25 mL of the test sample, and the eggs shall be incubated for 3 days. At least 20 other embryonated eggs aged 5–7 days shall be inoculated by the yolk sac route with 0.25 mL of the test sample, and the eggs shall be incubated for 7 days. No eggs shall show evidence of infection with extraneous viruses.

### 3.2 Tests on purified HBsAg suspension

#### 3.2.1 Test for HBsAg subtype

The test to identify HBsAg subtype shall be conducted serologically.

#### 3.2.2 Tests for purity of HBsAg suspension

The test sample and Reference HBsAg shall be used. The content of total protein shall be determined by the Test for Protein Content in General Tests, and that of HBsAg protein by radioimmunoassay or other appropriate methods. Alternatively, the specific HBsAg proteins and other extraneous proteins shall be determined by polyacrylamide gel electrophoresis or other appropriate methods. The content of HBsAg protein shall be equal to or not less than 95% of the total protein content.

#### 3.2.3 Test for freedom from hepatitis B virus DNA

The test sample containing 200 µg amount of HBsAg protein shall be used. Using <sup>32</sup>P-labelled hepatitis B virus DNA as the probe, the DNA-DNA hybridization test shall be performed under conditions allowing the detection of 1 pg of Reference Hepatitis B Virus DNA. No hepatitis B virus DNA shall be detected in the test sample.

### 3.3 Tests on bulk material

#### 3.3.1 Sterility test

The test given in General Tests shall apply.

#### 3.3.2 Inoculation of chimpanzees

A group of 2 or more healthy chimpanzees shall be used. The animals shall be free from hepatitis B virus antigens and antibodies to hepatitis B virus antigens, and have normal liver biopsies and normal levels of aminotransferases. Test sample containing 2 mg of HBsAg protein shall be given by intravenous injection into each animal, and the animals shall be observed for 6 months. The animals shall be proved to be free from hepatitis by histopathological and biochemical examinations, and from infection with hepatitis B virus by serological examinations. When 2 successive products pass this test, this test can be omitted in the bulk remaining untested.

### 3.4 Tests on final bulk

#### 3.4.1 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

#### 3.4.2 Sterility test

The test given in General Tests shall apply.

#### 3.4.3 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.5 Tests on final product

Following tests shall apply to each final lot.

#### 3.5.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 5.4 to 7.4.

#### 3.5.2 Test for aluminum content

When the test given in General Tests is applied, the aluminum content shall be no higher than 0.5mg/mL.

#### 3.5.3 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

#### 3.5.4 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%.

#### 3.5.5 Test for protein content

When either test given in methods 1) and 2) given below is applied, the protein content shall be no higher than 50 µg/mL.

Method 1) The test given in General Tests is modified for HB vaccine protein content evaluation. After the addition of 10 w/v% of trichloroacetic acid, the samples are heated for 15 minutes at 80°C in a water bath instead of boiling. During this assay, centrifugation shall be done at over 1900 × g. The alkaline-copper solution treatment takes 14–18 hours.

Method 2) Standard albumin for protein determination is accurately diluted with water to make standard dilutions of 10, 20, 40 and 60 µg/mL.

The test sample and standard dilutions are accurately taken in an amount of 0.5 mL, combined with 2 volumes of gel-solubilizer (0.4 mol/L sodium phosphate and 0.45 mol/L sodium citrate), and heated for 1 hour at 60°C. After cooling, 150 µL of 0.15% sodium deoxycholate solution is added to each sample and the mixture is incubated for 10 minutes at room temperature. Trichloroacetic acid is added to make the final concentration at 60mg/mL and the mixture is incubated for 1 hour on ice. After incubation, the mixture is centrifuged for 10 minutes at 10000 × g. To the resulting precipitates, 1 mL of 5 w/v% trichloroacetic acid is added. The mixture is shaken well and centrifuged again. This step is repeated twice.

The resulting precipitates are mixed with 0.5 mL of the SDS-alkaline copper solution and dissolved by shaking well. After dissolving, 0.5 mL of water is mixed and incubated for 20 minutes at room temperature. To the solution, 0.25 mL of the six-fold dilutions of Folin's test solution is added and the mixture is kept standing for 30 minutes at room temperature. The absorbance of the solution is measured at the wavelength of 750 nm with a spectrophotometer. If the solution is turbid, supernatant shall be collected and measured after centrifugation at more than 10,000 × g.

The values obtained with the standard dilutions are used to draw a calibration curve. The protein content of the test sample is calculated by interpolating the value obtained with the test sample to the calibration curve, and the protein content in 1 mL of the test sample is calculated from its content in the test dilution.

### 3.5.6 Sterility test

The test given in General Tests shall apply.

### 3.5.7 Test for freedom from abnormal toxicity

The test in General Tests shall apply.

### 3.5.8 Potency test

Potency shall be determined by detecting anti-HBs antibody produced in the immunized mice by the passive hemagglutination test or other appropriate method.

#### 3.5.8.1 Materials

The test sample and Reference Hepatitis B Vaccine (hereafter referred to as "Reference" in this monograph) shall be used. The diluent for the test sample and Reference shall be physiological saline.

#### 3.5.8.2 Test procedures

The test sample and the Reference shall be diluted to make appropriate logarithmic serial dilutions. At least 16 mice aged 5 weeks shall be used for each dilution. A dose of 1 mL per mouse shall be given by intraperitoneal or subcutaneous injection. The animals shall be bled 5 weeks after injection. Anti-HBs antibody in the serum of each animal shall be detected by the passive hemagglutination test or other appropriate method.

#### 3.5.8.3 Criterion for judgment

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

#### 3.5.9 Identity test

The test shall be conducted serologically using the Reference Hepatitis B Vaccine as reference.

#### 4. Storage and expiry date

The expiry date shall be two years.

#### 5. Other requirements

##### 5.1 Information to be provided in package insert and other labeling

- (1) HBsAg subtype contained in the product
- (2) Caution that the product be rendered homogenous by thorough shaking before use
- (3) Recommended human dose and route of administration, as follows:
  - 1) Prevention against hepatitis B: generally, 3 doses of 0.5 mL are given by subcutaneous or intramuscular injection at an interval of 4 weeks for the first 2 doses and 20–24 weeks after the first dose for the third dose. The dose may be reduced to 0.25 mL for children aged below 10 years.
  - 2) Prevention against transmission of hepatitis B virus from mother to child in the perinatal period (combined use with Human Anti-HBs Immunoglobulin): generally, 3 doses of 0.25 mL are given by subcutaneous injection, with the second and third doses injected at intervals of 1 month and 3 months after the first injection, respectively.
  - 3) Prevention against incidental transmission by HBeAg-positive and anti-HBsAg-positive blood (combined use with Human Anti-HBs Immunoglobulin): generally, the first dose of 0.5 mL is given by subcutaneous injection within 7 days after the accident, with the second and third doses injected at intervals of 1 month and 3–6 months after the first injection, respectively. The dose may be reduced to 0.25 mL for those aged below 10 years. Booster immunization may be provided to those who fail to produce active anti-HBs antibody.

## **ADSORBED HEPATITIS B VACCINE (PREPARED FROM huGK-14 CELLS)**

### **1. Descriptive definition**

“Adsorbed Hepatitis B vaccine (prepared from huGK-14 cells)” is a liquid product containing "hepatitis B surface antigen" (hereafter referred to as 'HBsAg' in this monograph), which is prepared from the human cell line, huGK-14 cells (hereafter referred to as “hu-GK-14 cells” in this monograph) and rendered insoluble by the addition of aluminum salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

### **2. Production control**

#### 2.1 Source materials

##### 2.1.1 Seed cell strain

The huGK-14 cell strain is established as HBsAg high-producing cells.

##### 2.1.2 Master cell bank

Master cell bank shall be dispensed after the cultivation of the seed cell strain under the fixed cultured condition within given passage numbers, and shall meet specifications required for the bank.

##### 2.1.3 Working cell bank

Working cell bank shall be dispensed after the cultivation of the seed cell strain under the fixed cultured condition within given passage numbers, and shall meet specifications required for the bank.

##### 2.1.4 Culture media

The medium suitable for the culture of huGK-14 cells shall be used.

#### 2.2 Bulk material

##### 2.2.1 HBsAg suspension

HBsAg suspension shall be the cultured and grown seed cells prepared from the working cell bank. The HBsAg suspension shall be free from contaminating bacteria by microscopic examination and appropriate culture.

##### 2.2.2 Purified HBsAg suspension

HBsAg shall be extracted and purified from the HBsAg suspension using suitable procedures. The bulk material shall contain purified HBsAg suspension.

#### 2.3 Final bulk

The bulk material shall be diluted in buffered physiological saline or other suitable medium, and supplemented with aluminum salt to serve as the final bulk. Appropriate preservatives may be added.

### **3. Control tests**

#### 3.1 Tests on the working cell bank

When working cell bank is prepared, the cell suspension shall be subjected to the tests given

below.

#### 3.1.1 Identification test on cultured huGK-14 cell strain

When huGK-14 cells are cultured with a suitable medium, the growth activity shall be normal.

#### 3.1.2 Identification test on HBsAg

When immunological method or other suitable method is applied, the production of HBsAg shall be normal.

### 3.2 Tests on bulk material

#### 3.2.1 Sterility test

The test given in General Tests shall apply.

#### 3.2.2 HBsAg polypeptide test

When the test sample treated by a suitable reducing-reagent is applied on HBsAg polypeptide tests on polyacrylamide gel electrophoresis following silver staining or other suitable staining method and Western blot method, the separated HBsAg polypeptides shall be normal.

#### 3.2.3 Tests for purity of HBsAg suspension

The specific HBsAg proteins and other extraneous proteins shall be determined by polyacrylamide gel electrophoresis, liquid-chromatography or other appropriate methods. The content of HBsAg protein shall be no less than 99% of the total protein.

#### 3.2.4 Cellular DNA test

When hybridization method as a probe of <sup>32</sup>P-labeled Alu-sequence DNA is applied the content of Cellular DNA shall be not be higher than 1 pg per 10 µg of HBsAg protein.

### 3.3 Tests on final product

Following tests shall apply to each final lot.

#### 3.3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 5.5 to 7.0.

#### 3.3.2 Test for aluminum content

When the test given in General Tests is applied, the aluminum content shall be no higher than 0.4 mg/mL.

#### 3.3.3 Test for thimerosal content

When thimerosal is used as preservative, its content shall be no higher than 0.012 w/v% according to the test given in General Tests.

#### 3.3.4 Test for protein content

When the protein content test given in “absorbed hepatitis B vaccine” is applied, the protein content shall be no higher than 30 µg/mL.

#### 3.3.5 Sterility test

The test given in General Tests shall apply.

#### 3.3.6 Test for freedom from abnormal toxicity

The test in General Tests shall apply.

#### 3.3.7 Potency test

Potency shall be determined by detecting the anti-HBs antibody produced in the immunized

mice by the passive hemagglutination test, enzyme immunoassay, or other appropriate methods.

#### 3.3.7.1 Materials

The test sample and Reference Hepatitis B Vaccine (hereafter referred to as "Reference" in this monograph) shall be used. The diluent for the test sample and the Reference shall be physiological saline.

#### 3.3.7.2 Test procedures

The test sample and the Reference shall be so diluted as to make appropriate logarithmic serial dilutions, respectively. At least 16 mice aged 5 weeks shall be used for each dilution. A dose of 1 mL shall be given by subcutaneous or intraperitoneal injection to each mouse. The animals shall be bled 5 weeks after immunizing injection. The anti-HBs antibody titers in the serum of each animal shall be determined by the passive hemagglutination test, enzyme immunoassay, or other appropriate methods.

#### 3.3.7.3 Criterion for judgment

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

#### 3.3.8 Identity test

The test shall be conducted serologically.

### **4. Storage and expiry date**

The expiry date shall be two years.

### **5. Other requirements**

#### 5.1 Information to be provided in package insert and other labeling

- (1) The HBsAg subtype contained in the product
- (2) Caution that the product be rendered homogenous by thorough shaking before use

# **RECOMBINANT ADSORBED HEPATITIS B VACCINE (PREPARED FROM YEAST)**

## **1. Descriptive definition**

“Recombinant Adsorbed Hepatitis B Vaccine (prepared from yeast)” is a liquid product containing "hepatitis B surface antigen" (hereafter referred to as 'HBsAg' in this monograph), which is prepared from the transformed yeast and rendered insoluble by adding aluminum salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

## **2. Production control**

### 2.1 Source material

#### 2.1.1 Master cell bank

A master cell bank shall be prepared from the selected clone of transformed yeast cells, which carry the yeast vector inserted with the structural gene coding for HBsAg by recombinant DNA technologies. The master cell bank shall be propagated in culture and dispensed.

#### 2.1.2 Working cell bank

A working cell bank shall be prepared by dispensing the cultured master cell bank. The working cell bank shall be subjected to the tests given in 3.1.

#### 2.1.3 Culture medium

A suitable culture medium shall be used for each transformed yeast.

### 2.2 Bulk material

#### 2.2.1 Yeast suspension

Yeast suspension shall be the cultured and grown seed cells from the working cell bank. The yeast suspension shall be free from contaminating bacteria by microscopic examination and appropriate culture methods.

#### 2.2.2 Purified HBsAg suspension

The HBsAg shall be extracted and purified from the yeast suspension using suitable procedures. The bulk material shall contain purified HBsAg suspension. The bulk material shall be subjected to the tests given in 3.2.

### 2.3 Final bulk

The bulk material shall be diluted in buffered physiological saline or other suitable medium, and supplemented with aluminum salt to serve as the final bulk. Appropriate preservatives may be added.

## **3. Control tests**

### 3.1 Tests on the working cell bank

When the working cell bank is prepared, the yeast suspension cultured from the working cell bank shall be subjected to the tests given below.

#### 3.1.1 Test on cultured yeast

When yeast is cultured with a suitable medium, the requirements for nutrition and growth activity shall be met.

#### 3.1.2 Test on HBsAg

When an immunological method or other suitable method is applied, the production of HBsAg shall be normal.

#### 3.1.3 Tests on vector and inserted gene

When vector recovered from the yeast is applied to fragmentation tests by restriction enzyme or other suitable tests, the vector and inserted gene shall be normal.

### 3.2 Tests on bulk material

#### 3.2.1 Sterility test

The test given in General Tests shall apply.

#### 3.2.2 HBsAg polypeptide test

When the test sample treated by a suitable reducing reagent is applied to HBsAg polypeptide tests on polyacrylamide gel electrophoresis following the silver staining or other suitable staining method and Western blot method, the separated HBsAg polypeptides shall be not abnormal.

#### 3.2.3 Tests for purity of HBsAg suspension

When polyacrylamide gel electrophoresis, liquid-chromatography or other appropriate methods are applied. The content of HBsAg protein shall be no less than 97.5% of the total protein.

### 3.3 Tests on final product

Following tests shall apply to each final lot.

#### 3.3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 5.5 to 8.0.

#### 3.3.2 Test for aluminum content

When the test given in General Tests is applied, the aluminum content shall be no higher than 0.65 mg/mL.

#### 3.3.3 Test for thimerosal content

When thimerosal is used as a preservative, the thimerosal content shall be no higher than 0.012 w/v% according to the test given in General Tests.

#### 3.3.4 Test for protein content

When the protein content test for absorbed hepatitis B vaccine is applied, the protein content shall be no higher than 35 µg/mL.

#### 3.3.5 Sterility test

The test given in General Tests shall apply.

#### 3.3.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.3.7 Potency test

Potency shall be determined by detecting anti-HBs antibody produced in immunized mice by the passive hemagglutination method, enzyme immunoassay, or other appropriate method.

##### 3.3.7.1 Materials

The test sample and Reference Hepatitis B Vaccine (hereafter referred to as "Reference" in this monograph) shall be used. The diluent for the test sample and Reference shall be physiological saline.

#### 3.3.7.2 Test procedures

The test sample and Reference shall be diluted to make appropriate logarithmic serial dilutions. At least 16 mice aged 5 weeks shall be used for each dilution. A dose of 1 mL shall be given by subcutaneous or intraperitoneal injection to each mouse. The animals shall be bled 5 weeks after immunizing injection and anti-HBs antibody titers in the serum of each animal shall be determined by the passive hemagglutination test, enzyme immunoassay, or other appropriate method.

#### 3.3.7.3 Criterion for judgment

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

#### 3.3.8 Identity test

The test shall be conducted serologically.

### **4. Storage and expiry date**

The expiry date shall be two years.

### **5. Other requirements**

#### 5.1 Information to be provided in package insert and other labeling

- (1) The HBsAg subtype contained in the product
- (2) Caution that the product be rendered homogenous by thorough shaking before use
- (3) Recommended human dose and route of administration, as follows:

Prevention against hepatitis B: generally, 3 doses of 0.5 mL are given by subcutaneous or intramuscular injection at an interval of 4 weeks for the first 2 doses and 20–24 weeks after the first dose for the third dose. The dose may be reduced to 0.25 mL for children aged below 10 years. Booster immunization may be provided to those who failed to acquire active anti-HBs antibody.

# **RECOMBINANT ADSORBED HEPATITIS B VACCINE (PREPARED FROM CHINESE HAMSTER OVARY CELLS)**

## **1. Descriptive definition**

“Recombinant Adsorbed Hepatitis B Vaccine (prepared from Chinese hamster ovary cells)” is a liquid product containing "hepatitis B surface antigen" (hereafter referred to as 'HBsAg' in this monograph), which is prepared from the transformed Chinese hamster ovary cells (hereafter referred to as 'CHO cells' in this monograph) and rendered insoluble by adding aluminum salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

## **2. Production control**

### 2.1 Source material

#### 2.1.1 Master cell bank

A master cell bank shall be prepared from the selected clone of transformed CHO cells, which carry the approved vector inserted with the structural gene coding for HBsAg by recombinant DNA technologies. The master cell bank shall be propagated in culture and dispensed.

#### 2.1.2 Working cell bank

Working cell bank shall be prepared by dispensing the cultured master cell bank. The working cell bank shall be subjected to the test given in 3.1.

#### 2.1.3 Culture media

A suitable culture medium shall be used for each recombinant CHO cells.

### 2.2 Bulk material

#### 2.2.1 HBsAg suspension

HBsAg suspension shall be the cultured and grown seed cells from the working cell bank. The HBsAg suspension shall be free from contaminating bacteria by microscopic examination and appropriate culture methods.

#### 2.2.2 Purified HBsAg suspension

HBsAg shall be extracted and purified from the HBsAg suspension using suitable procedures. The bulk material shall contain purified HBsAg suspension and shall be subjected to the tests given in 3.2.

### 2.3 Final bulk

The bulk material shall be diluted in buffered physiological saline or other suitable medium, and supplemented with aluminum salt to serve as the final bulk. Appropriate preservatives may be added.

## **3. Control tests**

### 3.1 Tests on the working cell bank

When the working cell bank is prepared, the cell suspension from the cultured working cell bank shall be subjected to the tests given below.

#### 3.1.1 Test on cultured CHO cells

When the CHO cells are cultured with a suitable medium, the growth activity shall be normal.

### 3.1.2 Test on HBsAg

When immunological method or other suitable method is applied, the production of HBsAg shall be normal.

## 3.2 Tests on bulk material

### 3.2.1 Sterility test

The test given in General Tests shall apply.

### 3.2.2 HBsAg polypeptide test

When the test sample treated by a suitable reducing reagent is applied to the HBsAg polypeptide tests on polyacrylamide gel electrophoresis following the silver staining or other suitable staining method and Western blot method, the separated HBsAg polypeptides shall be normal.

## 3.3 Tests on final product

Following tests shall apply to each final lot.

### 3.3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 5.5 to 7.5.

### 3.3.2 Test for aluminum content

When the test sample is allowed to form chelate complex with trans-1,2-cyclohexanediamine-N,N,N',N'-tetraacetic acid (CYDTA), and extra CYDTA is reverse-titrated with zinc solution, the aluminum content shall be no higher than 0.4 mg/mL.

### 3.3.3 Test for thimerosal content

When thimerosal is used as a preservative, the thimerosal content shall be no higher than 0.012 w/v% according to the test given in General Tests.

### 3.3.4 Test for protein content

When the protein content test for absorbed hepatitis B vaccine is applied, the protein content shall be no higher than 30 µg/mL.

### 3.3.5 Sterility test

The test given in General Tests shall apply.

### 3.3.6 Test for freedom from abnormal toxicity

The test in General Tests shall apply.

### 3.3.7 Potency test

Potency shall be determined by detecting anti-HBs antibody produced in immunized mice by the passive hemagglutination test, enzyme immunoassay, or other appropriate method.

#### 3.3.7.1 Materials

The test sample and Reference Hepatitis B Vaccine (hereafter referred to as "Reference" in this monograph) shall be used. The diluent for the test sample and Reference shall be physiological saline.

#### 3.3.7.2 Test procedures

The test sample and Reference shall be diluted to make appropriate logarithmic serial dilutions. At least 16 mice aged 5 weeks shall be used for each dilution. A dose of 1 mL shall be given by

subcutaneous or intraperitoneal injection to each mouse. The animals shall be bled 5 weeks after immunizing injection and anti-HBs antibody titers in the serum of each animal shall be determined by the passive hemagglutination test, enzyme immunoassay, or other appropriate method.

#### 3.3.7.3 Criterion for judgment

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

#### 3.3.8 Identity test

The test shall be conducted serologically.

### **4. Storage and expiry date**

The expiry date shall be two years.

### **5. Other requirements**

#### 5.1 Information to be provided in package insert and other labeling

- (1) The HBsAg subtype contained in the product
- (2) Caution that the product be rendered homogenous by thorough shaking before use

**RECOMBINANT ADSORBED HEPATITIS B VACCINE CONTAINING pre-S2  
ANTIGEN AND HBs ANTIGEN  
(PREPARED FROM YEAST)**

**1. Descriptive definition**

“Recombinant Adsorbed Hepatitis B Vaccine Containing pre-S2 Antigen and HBs Antigen (prepared from yeast)” is a liquid product containing "hepatitis B virus surface antigen,” of which one molecule contains pre-S2 antigen and HBs antigen (hereafter referred to as 'pre-S2•HBsAg' in this monograph), and which is prepared from the recombinant yeast and rendered insoluble by adding aluminum salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

**2. Production control**

2.1 Source material

2.1.1 Master cell bank

A master cell bank shall be prepared from the selected clone of transformed yeast cells, which carry the yeast expression vector inserted with the structural gene coding for pre-S2•HBsAg by recombinant DNA technologies. The master cell bank shall be propagated in cell culture and dispensed. The bulk material shall be structured from the use of master cell bank.

The master cell bank shall be subjected to the tests given in 3.1

2.1.2 Culture medium

A suitable culture medium shall be used for each recombinant yeast.

2.2 Bulk material

2.2.1 Yeast suspension

Yeast suspension shall be the cultured and grown seed cells from the working cell bank. The yeast suspension shall be confirmed free from other contaminated bacterium by microscopic examination and other appropriate culture methods.

2.2.2 Purified HBsAg suspension

The pre-S2•HBsAg shall be extracted and purified from the yeast suspension using suitable procedures. The bulk material shall contain purified pre-S2•HBsAg suspension. The bulk material shall be subjected to the tests given in 3.2.

2.3 Final bulk

The bulk material shall be diluted in buffered physiological saline or other suitable medium, and supplemented with aluminum salt to serve as the final bulk. Appropriate preservatives may be added.

**3. Control tests**

3.1 Tests on the working cell bank

When the working cell bank is prepared or is under storage, if necessary, the yeast suspension cultured from the working cell bank shall be subjected to the tests given below.

3.1.1 Tests for cultured yeast

When yeast is cultured with a suitable medium, the requirements for nutrition and growth activity shall be normal.

#### 3.1.2 Test for pre-S2•HBsAg

When an immunological method or other suitable method is applied, the production of pre-S2•HBsAg shall be normal.

#### 3.1.3 Tests for vector and inserted gene

When expression plasmid recovered from the yeast is applied to fragmentation tests by restriction enzyme or other suitable tests, the vector and inserted gene shall be normal.

### 3.2 Tests on bulk material

#### 3.2.1 Sterility test

The test given in General Tests shall apply.

#### 3.2.2 pre-S2•HBsAg polypeptide test

When the test sample treated with a suitable reducing reagent is applied to pre-S2•HBsAg polypeptide tests on polyacrylamide gel electrophoresis following the silver staining or other suitable staining method and Western blot method, the separated pre-S2•HBsAg polypeptides shall be normal.

#### 3.2.3 Tests for purity of HBsAg suspension

When polyacrylamide gel electrophoresis, liquid chromatography or other appropriate methods are applied, the content of pre-S2•HBsAg protein shall be no less than 97.5% of the total protein.

### 3.3 Tests on final product

Following tests shall apply to each final lot.

#### 3.3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.0 to 7.0.

#### 3.3.2 Test for aluminum content

When the test given in General Tests is applied, the aluminum content shall be no higher than 0.5 mg/mL.

#### 3.3.3 Test for thimerosal content

When thimerosal is used as a preservative, the thimerosal content shall be no higher than 0.012 w/v% according to the test given in General Tests.

#### 3.3.4 Test for protein content

When the protein content test for absorbed hepatitis B vaccine is applied, protein content shall be no higher than 50 µg/mL.

#### 3.3.5 Sterility test

The test given in General Tests shall apply.

#### 3.3.6 Test for freedom from abnormal toxicity

The test in General Tests shall apply.

#### 3.3.7 Potency test

Potency shall be determined by detecting anti-pre-S2 and HBs antibody produced in immunized mice by the passive hemagglutination test, enzyme immunoassay, or other appropriate method.

#### 3.3.7.1 Materials

The test sample and Reference Hepatitis B Vaccine (hereafter referred to as "Reference" in this monograph) shall be used. The diluent for the test sample and Reference shall be physiological saline.

#### 3.3.7.2 Test procedures

##### (1) HBs antibody:

The test sample and Reference shall be diluted to make appropriate logarithmic serial dilutions. At least 16 mice aged 5 weeks shall be used for each dilution. A dose of 1 mL shall be given by subcutaneous or intraperitoneal injection to each mouse. The animals shall be bled 5 weeks after immunizing injection, and anti-HBs antibody titers in the serum of each animal shall be detected by the passive hemagglutination method, enzyme immunoassay, or other appropriate method.

##### (2) pre-S2 antibody:

The test sample and Reference shall be diluted to make appropriate logarithmic serial dilutions. At least 16 mice aged 5 weeks shall be used for each dilution. A dose of 1 mL shall be given by subcutaneous or intraperitoneal injection to each mouse. The animals shall be bled 5 weeks after immunizing injection, and anti-pre-S2 antibody titers in the serum of each animal shall be determined by the passive hemagglutination test or other appropriate method.

#### 3.3.7.3 Criterion for judgment

##### (1) HBs antibody:

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

##### (2) pre-S2 antibody:

When  $ED_{50}$  per mL of the test sample is calculated by statistical analysis of test results with 15 non-inoculated control serum samples, the  $ED_{50}$  value shall be no less than 40.

#### 3.3.8 Identity test

The test shall be conducted serologically.

### **4. Storage and expiry date**

The expiry date shall be two years.

### **5. Other requirements**

#### 5.1 Information to be provided in package insert and other labeling

- (1) The HBsAg subtype contained in the product
- (2) Caution that the product be rendered homogenous by thorough shaking before use

## **FREEZE-DRIED BCG FOR INTRAVESICAL USE (CONNAUGHT STRAIN)**

### **1. Descriptive definition**

“Freeze-dried BCG for Intravesical Use (Connaught strain)” is a freeze-dried product containing live bacteria derived from a culture from Calmette and Guerin (Connaught strain). When reconstituted, it becomes a whitish or pale yellowish turbid liquid.

### **2. Production control**

#### 2.1 Source materials

##### 2.1.1 Seed lot

The primary seed lot of the bacillus of Calmette and Guerin (Connaught strain) shall be cultured and the cultures shall be harvested to serve as the freeze-dried products of the secondary seed lot.

The secondary seed lot shall be subjected to the test given in 3.1, and if passed shall be stored at -20°C or less.

##### 2.1.2 Medium for cultivation

The medium specified in 2.2 shall be used.

#### 2.2 Bacterial growth for production

##### 2.2.1 Seed culture

The secondary seed lot shall be suspended in Sauton’s medium, inoculated on potato medium with glycerin solution, and cultivated at  $37 \pm 1^\circ\text{C}$  for 14 days. The growing pellicle shall be used as the seed culture.

##### 2.2.2 Cultivation and harvesting

The seed culture shall be inoculated on Sauton's medium and incubated at  $37 \pm 1^\circ\text{C}$  for 7–10 days. This cultivation shall be repeated 3 times. The pellicle growing on the surface of the medium shall be harvested by filtration or other suitable method.

The number of serial transfers from the primary seed lot until use for bulk production shall not exceed seven.

At the completion of cultivation, the medium shall be subjected to the test given in 3.2.

##### 2.2.3 Treatment of bacterial growth

Bacterial growth shall be triturated homogeneously by an appropriate method and suspended in a 1.5-w/v% sodium glutamate solution.

#### 2.3 Final bulk and dispensation

The bacterial suspension shall be used to adjust the moist bacterial concentration to 95 mg/mL and sodium glutamate solution to 5 w/v% to serve as the final bulk.

The final bulk shall be dispensed into final containers at the volume of 3.6 mL and freeze-dried. 81 mg of dried bacteria per container shall be distributed as the final product.

### **3. Control tests**

### 3.1 Test on seed lot

Secondary seed lots shall be subjected to the tests given below.

#### 3.1.1 Test for freedom from virulent mycobacteria

The test given in 3.4.8 shall apply. At least 12 guinea pigs shall be used, and observation shall be for more than 6 months. When more than one-third of animals die as a result of non-progressive tuberculous disease, a retest shall be conducted.

### 3.2 Test on medium at the completion of cultivation

The medium shall be clear at the completion of cultivation.

### 3.3 Test on final bulk

#### 3.3.1 Sterility test

The test given in General Tests shall apply. 1 mL of the test sample shall be inoculated on 100 mL of liquid medium into each of 10 tubes of liquid thioglycolate medium and soybean casein digest medium.

### 3.4 Test on final product

Following tests shall apply to each final lot.

#### 3.4.1 Test for moisture content

Moisture content shall be no higher than 3.4% when tested according to the test given in General Tests.

#### 3.4.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.2.

#### 3.4.3 Test for osmotic pressure ratio

The reconstituted test sample shall be diluted by the addition of 40mL physiological saline. The test given in General Tests in the JP shall apply, and the value shall be within the range of 1.0 to 1.2.

#### 3.4.4 Test for staining

Smears may be made directly from undiluted suspensions or from diluted specimens in physiological saline. Smears shall be stained by Gram's procedure and the acid-fast procedure, and examined microscopically to verify the morphological appearance of the bacteria.

#### 3.4.5 Sterility test

The test given in General Tests shall apply. From 20 selected containers, 1-mL portions of each test sample per container shall be inoculated into 1 tube per portion with 80mL medium per culture.

#### 3.4.6 Test for total bacterial content

The test given in General Tests in the JP is applied. Weight of the content shall be no more than 250mg.

#### 3.4.7 Potency test

Potency shall be determined by the method for counting the number of culturable particles.

##### 3.4.7.1 Materials

The contents of 6 final products shall be reconstituted in accordance with the description on the label, and then diluted  $2 \times 10^6$  times using Sauton's liquid medium containing 0.025% polysorbate 80 as the test solution. The test solution shall be further diluted two-fold as the  $1/2$  test solution ( $4 \times 10^6$

dilution).

#### 3.4.7.2 Test procedures

Each test dilution and 1/2 test dilution shall be inoculated on 10 Lowenstein-Jensen slopes at a dose of 0.1 mL. These cultures shall be incubated at  $36 \pm 2^\circ\text{C}$  for 4 weeks, and the number of colonies appearing per culture tube shall be counted. Using the value of each tube, the number of colony forming units per vial shall be calculated.

When the tests meet the requirements described below, the results obtained can be applied to the calculation of colony forming units

- (1) Ratio of mean colony forming units from two dilutions shall be within the range of 1.30 to 2.70.
- (2) The number of colonies appearing shall be within the range of 5 to 50 per culture tube.

#### 3.4.7.3 Criterion for judgment

The test may be accepted when the geometric mean for colony forming units per vial is within the range of  $1.8 \times 10^8$  to  $19.2 \times 10^8$ . When the number of corresponding vials is less than 5, a retest shall be conducted. When the results do not meet the specification, the test can be repeated once. Potency shall be judged by the overall results, including those of the first test.

#### 3.4.8 Test for freedom from virulent mycobacteria

Each vial shall be reconstituted with 3mL of diluent. The solution from each vial shall be pooled, and the pooled preparation shall be diluted 1/13.3 for injection into animals. Each of 7 tuberculin-negative guinea pigs weighing 250–400 g shall be given by subcutaneous injection into the groin with 2 mL of diluted solution. The animals shall be observed at least 6 weeks. At the end of this period, all animals shall be examined by post-mortem for evidence of virulent mycobacterial lesions. No animal shall show any abnormal signs, except lesions with an apparent tendency toward recovery at the site of injection and regional lymph nodes. No animal shall show progressive tuberculous lesions or other abnormal changes, except mild lesions with a tendency toward recovery at autopsy performed at the end of the observation period. For the test to be valid, if more than 3 guinea pigs die during the observation period, the test shall be repeated using 6 guinea pigs. In the second test, if more than 2 animals fail to survive the observation period, the test shall be rejected.

#### 3.4.9 Identity test

The test shall be conducted by microscopic examination of stained specimens.

### 4. Storage and expiry date

The expiry date shall be two years during storage at  $2-8^\circ\text{C}$ .

### 5. Other requirements

#### 5.1 Final containers

Colored containers conforming to the requirements given in the test of glass containers for injections of the JP shall be used. The containers shall be sealed with a rubber stopper.

##### 5.1.1 Light shield test

The light shield test for glass vials shall be conducted according to the requirements given in the test of colored containers for injections of the JP. Permeability shall be no more than 50% at

290–450 nm.

## 5.2 Reconstituent

The reconstituent shall be phosphate-buffered sodium chloride solution containing polysorbate 80.

## **FREEZE-DRIED BCG FOR INTRAVESICAL USE (JAPANESE STRAIN)**

### **1. Descriptive definition**

“Freeze-dried BCG for Intravesical Use (Japanese strain)” is a freeze-dried product containing live bacteria derived from a culture from Calmette and Guerin, Japanese strain. When reconstituted, it becomes a whitish or pale yellowish turbid liquid.

### **2. Production control**

#### 2.1 Source materials

##### 2.1.1 Strain used for production

The strain of bacteria supplied by the National Institute of Infectious Diseases shall be used for production. The strain for production shall be allowed to be serially cultivated on Sauton's potato medium or calf-bile potato medium at  $37.5 \pm 0.5^\circ\text{C}$  at intervals of about 2 weeks, and kept at  $5^\circ\text{C}$  or less between the periods of cultivation. The BCG strain licensed for the production shall not be passaged more than 12 times.

##### 2.1.2 Medium for cultivation

The medium specified in 2.2 shall be used.

#### 2.2 Bacterial growth for production

##### 2.2.1 Seed culture

The strain for production shall be inoculated on potato medium with glycerin solution, Sauton's potato medium or Sauton's medium, and cultivated at  $37.5 \pm 0.5^\circ\text{C}$ . The culture can be repeated only once within a period of 3 weeks, if necessary.

The bacterial growth after 5 days' cultivation shall be inoculated to Sauton's medium, cultured at  $37.5 \pm 0.5^\circ\text{C}$  for 7–10 days, and the growing pellicle shall be used as the seed culture.

##### 2.2.2 Cultivation and harvesting

The seed culture shall be inoculated on Sauton's medium and incubated at  $37.5 \pm 0.5^\circ\text{C}$  for 7–10 days. The pellicle growing on the surface of medium shall be harvested by filtration or by other suitable method. The pellicle shall cover the entire surface area of medium and shall be judged as being at the stage of active growth.

At the completion of cultivation, the medium shall be subjected to the test given in 3.1.

##### 2.2.3 Treatment of bacterial growth

Bacterial growth shall be treated by an appropriate method to render the moisture content to approximately 70% and to serve as the bacteria for growth (hereafter referred to as "moist bacteria" in this monograph).

#### 2.3 Final bulk and dispensation

The moist bacteria shall be triturated homogeneously, and suspended in a solution of sodium glutamate at not higher than 15 w/v% to adjust the moist bacterial concentration to 80 mg/mL to serve as the final bulk. The solvent of the suspending solution shall be water for injection. The final bulk shall be subjected to the tests given in 3.2.

The final bulk shall be dispensed into final containers at the volume of 0.5 or 1.0 mL, freeze-dried and hermetically or tightly sealed at a pressure not higher than 13.3 Pa.

### **3. Control tests**

#### **3.1 Test on medium at completion of cultivation**

The medium shall be clear at the completion of cultivation.

#### **3.2 Control tests on final bulk**

##### **3.2.1 Test for staining**

Smears may be made directly from undiluted suspensions or from diluted specimens in physiological saline. Smears shall be stained by Gram's procedure and the acid-fast procedure, and examined microscopically to verify the morphological appearance of the bacteria.

##### **3.2.2 Sterility test**

The test given in General Tests shall apply.

##### **3.2.3 Test for freedom from virulent mycobacteria**

The test sample shall be diluted in physiological saline to render the content of moist bacteria to 2.5 mg/mL. The dilution shall be injected into at least 6 guinea pigs weighing 300–400 g and showing evidence of tuberculin negative tuberculosis within a week prior to the test.

A dose of 1 mL of the dilution shall be given by an intramuscular injection into the thigh of at least 3 animals and by a subcutaneous injection into the abdomen of at least 3 animals. The inoculated animals shall be observed for at least 6 weeks.

No animal shall show any abnormal signs, except lesions with an apparent tendency toward recovery at the site of injection and regional lymph nodes. No animal shall show progressive tuberculous lesions or other abnormal changes, except mild lesions with a tendency toward recovery at autopsy performed at the end of the observation period. At least two-third of the animals must survive the observation period.

#### **3.3 Tests on final product**

Following tests shall apply to each final lot.

##### **3.3.1 Test for moisture content**

Moisture content shall be no higher than 3.4% when tested according to the test given in General Tests.

##### **3.3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 5.5 to 7.0.

##### **3.3.3 Test for osmotic pressure ratio**

A 2-mL portion of the reconstituted test sample at the concentration of 40 mg/mL shall be diluted in 39 mL of physiological saline. When tested according to General Testing Methods in the JP, the value shall be within the range of 1.05 to 1.16.

##### **3.3.4 Staining test**

Smears may be made directly from undiluted suspensions or from diluted specimen in physiological saline. Smears shall be stained by Gram's procedure and the acid-fast procedure, and examined microscopically to verify the morphological appearance of the bacteria.

### 3.3.5 Sterility test

The test given in General Tests shall apply, and 0.25 mL of sample per container shall be inoculated into one tube each with 15mL medium per culture.

### 3.3.6 Test for total bacterial content

The test sample shall be diluted in physiological saline to render the content of moist bacteria to 1 mg/mL, and the absorbance of the dilution shall be measured by a spectrophotometer at 470 nm and light path of 10mm. The absorbance shall be no more than the determined value for each spectrophotometer.

### 3.3.7 Potency test

Potency shall be determined by the method for number of culturable particles.

#### 3.3.7.1 Materials

The contents of 10 final products shall be reconstituted in accordance with the description on the label, and further diluted in sterile water to a concentration of  $0.5 \times 10^{-4}$  mg moist bacteria per mL.

#### 3.3.7.2 Test procedures

Each dilution shall be inoculated to Ogawa's medium at a dose of 0.1 mL. The cultures shall be incubated at  $37.5 \pm 0.5^\circ\text{C}$  for 4 weeks, and the number of appearing colonies per culture tube shall be counted. Then, sum and unbiased variance of the square root of the values shall be calculated.

#### 3.3.7.3 Criterion for judgment

Using the value of each tube ( $n=10$ ), mean shall be no more than 381. The number of sum and unbiased variance of square root contained in the test sample shall be within a range elsewhere specified upon statistical analysis.

### 3.3.8 Identity test

The test shall be conducted by microscopic examinations of stained specimens.

## 4. Storage and expiry date

The expiry date shall be two years.

## 5. Other requirements

### 5.1 Final containers

Colored and hermetically sealed containers conforming to the requirements given in the test of glass containers for injections of the JP shall be used.

### 5.2 Reconstituent

The reconstituent shall be physiological saline.

## **FREEZE-DRIED BCG VACCINE (FOR PERCUTANEOUS USE)**

### **1. Descriptive definition**

“Freeze-dried BCG Vaccine (for percutaneous use)” is a freeze-dried product containing live bacteria derived from a culture of the bacillus of Calmette and Guerin (hereafter referred to as "bacteria" in this monograph). When reconstituted, it becomes a whitish or pale yellowish turbid liquid.

### **2. Production control**

#### 2.1 Source materials

##### 2.1.1 Strain used for production

The strain of bacteria delivered from the National Institute of Infectious Diseases shall be used for production. The strain for production is allowed to be serially cultivated on Sauton's potato medium or calf-bile potato medium at  $37.5 \pm 0.5^\circ\text{C}$  at intervals of about 2 weeks, and kept at  $5^\circ\text{C}$  or less between the period of cultivation. The BCG strain licensed for the production shall not be passaged more than 12 times.

##### 2.1.2 Medium used for cultivation

The medium listed in 2.2 shall be used.

#### 2.2 Bacterial growth for vaccine

##### 2.2.1 Seed culture

The strain for production shall be inoculated on potato medium with glycerin solution, on Sauton's potato medium or on Sauton's medium, and cultivated at  $37.5 \pm 0.5^\circ\text{C}$ . The culture can be repeated only once again within a period of 3 weeks, if necessary.

The bacterial growth after 5 days' cultivation shall be inoculated to Sauton's medium, cultured at  $37.5 \pm 0.5^\circ\text{C}$  for 7–10 days, and the growing pellicle shall be used as the seed culture.

##### 2.2.2 Cultivation and harvesting

The seed culture shall be inoculated on Sauton's medium and incubated at  $37.5 \pm 0.5^\circ\text{C}$  for 7–10 days. The pellicle growing on the surface of the medium shall be harvested by filtration or by other suitable method. The pellicle shall cover the whole surface area of medium and shall be judged as being at the stage of active growth.

At the completion of cultivation, the medium shall be subjected to the test given in 3.1.

##### 2.2.3 Treatment of bacterial growth

The bacterial growth shall be treated by an appropriate method to render the moisture content to approximately 70% and to serve as the bacteria for growth (hereafter referred to as "moist bacteria" in this monograph).

#### 2.3 Final bulk and dispensing

The moist bacteria shall be triturated homogeneously, and suspended in a solution of sodium glutamate at not higher than 15 w/v% to make the moist bacterial concentration to 80 mg/mL to serve as the final bulk. The solvent of the suspending solution shall be water for injection.

The final bulk shall be subjected to the tests given in 3.2. The final bulk shall be dispensed to

final containers at the volume of 0.15, 0.5 or 1.0 mL, freeze-dried and hermetically or tightly sealed at a pressure not higher than 13.3 Pa.

### **3. Control tests**

#### **3.1 Test on medium at completion of cultivation**

The medium shall be clear at the completion of cultivation.

#### **3.2 Tests on final bulk**

##### **3.2.1 Staining test**

Smears may be made directly from undiluted suspensions or from diluted specimens in physiological saline. Smears shall be stained by Gram's procedure and the acid-fast procedure, and examined microscopically to verify the morphological appearance of the bacteria.

##### **3.2.2 Sterility test**

The test given in General Tests shall apply.

##### **3.2.3 Test for freedom from virulent mycobacteria**

The test sample shall be diluted in physiological saline to render the content of moist bacteria to 2.5 mg/mL. The dilution shall be injected into at least 6 guinea pigs weighing 300–400 g and showing evidence of tuberculin-negative tuberculosis within a week prior to the test. A dose of 1 mL of the dilution shall be given by an intramuscular injection into the thigh of at least 3 animals and by a subcutaneous injection into the abdomen of at least 3 animals. The inoculated animals shall be observed for at least 6 weeks.

No animal shall show any abnormal signs, except lesions with apparent tendency toward recovery at the site of injection and regional lymph nodes. No animal shall show progressive tuberculous lesions or other abnormal changes, except mild lesions with a tendency toward recovery at autopsy performed at the end of the observation period. At least two-third of the animals must survive the observation period.

#### **3.3 Tests on final product**

Following tests shall apply to each final lot.

##### **3.3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

##### **3.3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 5.5 to 7.0.

##### **3.3.3 Staining test**

Smears may be made directly from undiluted suspensions or from diluted specimen in physiological saline. Smears shall be stained by Gram's procedure and the acid-fast procedure, and examined microscopically to verify the morphological appearance of the bacteria.

##### **3.3.4 Sterility test**

The test given in General Tests shall apply, and 0.25 mL of sample per container shall be inoculated into one tube each with 15 mL medium per culture. In the case the product is for individual use only, the amount of inoculation shall be the whole volume of container.

### 3.3.5 Test for total bacterial content

The test sample shall be diluted in physiological saline to render the content of moist bacteria to 1 mg/mL, and the absorbance of the dilution shall be measured by a spectrophotometer at 470 nm and light path of 10 mm. The absorbance shall be no more than the determined value for each spectrophotometer.

### 3.3.6 Potency test

Potency shall be determined by the method for number of culturable particles.

#### 3.3.6.1 Materials

The contents of 10 final products shall be reconstituted in accordance with the description on the label, and further diluted in sterile water to a concentration of  $0.5 \times 10^{-4}$  mg moist bacteria per mL.

#### 3.3.6.2 Test procedures

Each dilution shall be inoculated to Ogawa's medium at a dose of 0.1 mL. The cultures shall be incubated at  $37.5 \pm 0.5^\circ\text{C}$  for 4 weeks, and the number of colonies developed in one culture tube shall be counted. Then, sum and unbiased variance of the square root of the value shall be calculated.

#### 3.3.6.3 Criterion for judgment

The number of sum and unbiased variance of square root contained in the test sample shall be within a range elsewhere specified up upon statistical analysis.

### 3.3.7 Identity test

The test shall be conducted by microscopic examinations of stained specimens.

## 4. Storage and expiry date

The expiry date shall be two years.

## 5. Other requirements

### 5.1 Final containers

Colored and hermetically sealed containers conforming to the requirements given in the test of glass containers for injections of the JP shall be used.

### 5.2 Labeling

The description "for percutaneous use only" shall be provided on the label. Additionally, the description "individual use only" shall be provided on the label of the product for individual use.

### 5.3 Reconstituent

The reconstituent shall be physiological saline.

### 5.4 Information to be provided in package insert and other labeling

Recommended human dose and route of administration, as follows:

A drop of the reconstituted product shall be topically applied to the center of outer side of the upper arm, and inoculated by the needle for percutaneous application.

# PERTUSSIS VACCINE

## 1. Descriptive definition

“Pertussis Vaccine” is a whitish turbid liquid product containing inactivated *Bordetella pertussis* (hereafter referred to as "bacteria" in this monograph).

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Strains of bacteria

Strains of phase I *B. pertussis* shall be used.

#### 2.1.2 Culture medium

The media used for cultivation of bacteria shall not contain any impurities, which have the possibility of inducing pronounced allergic reactions in human.

### 2.2 Bulk material

#### 2.2.1 Suspension of bacteria

The cultured bacteria shall be tested microscopically and by an appropriate culture method. When no contaminant is detected, the bacterial suspension shall serve as a single-strain suspension.

#### 2.2.2 Inactivation and detoxification

Inactivation and detoxification shall be conducted by heating, addition of thimerosal or other appropriate method. After the completion of inactivation and detoxification, the suspension shall serve as a single-strain bulk.

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

### 2.3 Final bulk

The single-strain bulk shall be diluted with buffered physiological saline or other suitable medium and mixed, if necessary, to make the bacterial content not higher than  $2.0 \times 10^{10}$  per mL when determined by the test given in 3.1.2.

Appropriate preservatives may be added.

The final bulk shall be subjected to the tests given in 3.2.

## 3. Control tests

### 3.1 Tests on single-strain bulk

#### 3.1.1 Staining test

The test given in General Tests shall apply.

#### 3.1.2 Test for bacterial content

The test sample shall be checked by the optical density test given in General Tests before being subjected to any treatment given in 2.2.2. The optical density of test material containing  $1.0 \times 10^{10}$  of bacteria per mL shall correspond to 10 opacity units.

#### 3.1.3 Agglutination test

The test sample shall be diluted adequately and tested for agglutinability. The organisms shall be agglutinated by an antiserum against the heat-labile agglutigen (K factor), and hardly agglutinated by an antiserum against the heat-stable agglutigen (O factor) of bacteria.

#### 3.1.4 Sterility test

The test given in General Tests shall apply.

### 3.1.5 Inactivation test

When the test sample is cultured on blood agar medium or other suitable medium, no growth of bacteria shall be observed.

### 3.1.6 Test for freedom from heat-labile toxin

The test sample shall be diluted with 0.013 mol/L phosphate-buffered sodium chloride solution (pH 7.0) to render the bacterial content no lower than 10 times that of the final bulk.

The diluted test sample shall be given by intracutaneous injection at a dose of 0.025 mL into at least 4 suckling mice aged 48–72 hours or into at least 2 rabbits weighing 2.0 to 4.0 kg at a dose of 0.1 mL. The inoculated animals shall be observed for 4 days. No animal shall show any local lesion due to the heat-labile toxin of bacteria.

### 3.1.7 Test for mouse leukocytosis-promoting toxicity

The test sample shall be diluted with 0.013 mol/L phosphate-buffered sodium chloride solution (pH 7.0) to render the bacterial content equal to that of the final bulk. The diluted test sample shall be given by intraperitoneal injection at a dose of 0.5 mL into at least 5 mice aged 4 weeks. The mean count of leukocytes in peripheral blood 3 days after injection shall not exceed 10 times that before injection.

## 3.2 Tests on final bulk

### 3.2.1 Test for thimerosal content

When thimerosal has been added as a preservative, the test given in General Tests shall apply. The thimerosal content shall be no higher than 0.012 w/v%.

### 3.2.2 Staining test

The test given in General Tests shall apply.

### 3.2.3 Sterility test

The sterility test given in General Tests shall apply.

### 3.2.4 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.2.5 Test for toxicity to mouse weight gain

The test given in 3.3.8 shall apply.

## 3.3 Tests on final product

Following tests shall apply to each final lot.

### 3.3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

### 3.3.2 Test for protein nitrogen content

When the test given in General Tests is applied, the protein nitrogen content shall be no higher than 110 µg/mL.

### 3.3.3 Test for thimerosal content

When thimerosal has been added as a preservative, the test given in General Tests shall apply. The thimerosal content shall be no higher than 0.012 w/v%.

### 3.3.4 Test for formaldehyde content

When formaldehyde is used for the inactivation and detoxification of bacteria, the test sample shall be subjected to the test given in General Tests. The formaldehyde content shall be no higher than 0.01 w/v%.

### 3.3.5 Test for bacterial content

When the test for optical density given in General Tests is applied, the opacity shall be not more

than 20 opacity units.

#### 3.3.6 Sterility test

The sterility test given in General Tests shall apply.

#### 3.3.7 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.3.8 Test for toxicity to mouse weight gain

The test sample shall be given by intraperitoneal injection into at least 5 mice aged 5 weeks at a dose of 0.3 mL. The inoculated animals shall be observed for 7 days. Mean body weight 3 days after injection shall be no less than that at the time of injection upon statistical analysis. No animal shall show any abnormal signs during the observation period.

#### 3.3.9 Test for mouse leukocytosis-promoting toxicity

The test sample shall be given by intraperitoneal injection at a dose of 0.5 mL into at least 5 mice aged 4 weeks. The mean count of leukocytes in peripheral blood 3 days after injection shall not exceed 10 times that before injection.

#### 3.3.10 Potency test

Potency shall be determined in mice by the intracerebral challenge method.

##### 3.3.10.1 Materials

The test sample, Standard Pertussis Vaccine (hereafter referred to as "Standard" in this monograph) and strain 18323 of *B. pertussis* for challenge or other strains with equivalent character (hereafter referred to as "challenge strain" in this monograph) shall be used. The diluent for the test sample and Standard shall be 0.013 mol/L phosphate-buffered sodium chloride solution (pH 7.0). The challenge strain shall be cultured on Bordet-Gengue blood agar medium for about 24 hours and suspended in 0.6 w/v% sodium chloride solution containing 1 w/v% casamino acid solution (pH 7.0–7.2) to a concentration of approximately 200 LD<sub>50</sub> per 0.025 mL to serve as the suspension for challenge (hereafter referred to as "challenge suspension" in this monograph).

##### 3.3.10.2 Test procedures

The test sample and Standard shall be diluted to make at least three levels of four-fold or other suitable logarithmic serial dilutions, respectively. Each dilution shall be given by intraperitoneal injection at a dose of 0.5 mL into at least 16 mice aged 4 weeks. The animals shall be of the same sex or both sexes in equal numbers. At 14 days after immunizing injection, the challenge suspension shall be given by intracerebral injection into the animals at a dose of 0.025 mL. The animals shall be observed for 14 days. Any animal dying within 3 days after challenge shall be excluded from the test. Any animal showing paralysis or swelling of the head at the end of the observation period shall be included in deaths. Each of at least three appropriate serial dilutions of the challenge suspension shall be injected into at least 10 mice aged 6 weeks to titrate the virulence. The bacterial count for the LD<sub>50</sub> per 0.025 mL of the challenge suspension shall be 50–400 cells. The live cell count in the challenge suspension obtained by culture on Bordet-Gengue blood agar medium shall be approximately one-fourth of the total bacterial count obtained in the opacity test given in 3.1.2.

##### 3.3.10.3 Criterion for judgment

The potency of the test sample shall be no less than 8 IU/mL upon statistical analysis of the results with those of the International Standard.

#### 3.3.11 Identity test

The test shall be conducted by agglutination in tubes with immune serum against *B. pertussis*

#### **4. Storage and expiry date**

The expiry date shall be 18 months.

#### **5. Other requirements**

5.1 Information to be provided in package insert and other labeling

- (1) Name of the strains used for production
- (2) Method used for the inactivation of bacteria
- (3) Caution that the product be rendered homogenous by thorough shaking before use
- (4) Recommended human dose and route of administration, as follows:

For primary immunization, usually 3 doses of 0.5 mL are given by subcutaneous injection at intervals of 3–8 weeks.

For booster immunization, usually 0.5 mL is given by subcutaneous injection with an interval of not less than 6 months after completion of the primary immunization (in general, between 12 to 18 months after completion of the primary immunization).

## ADSORBED PURIFIED PERTUSSIS VACCINE

### 1. Descriptive definition

“Adsorbed Purified Pertussis Vaccine” is a liquid product containing protective antigen(s) of *Bordetella pertussis* and is rendered insoluble by adding aluminum salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

### 2. Production control

#### 2.1 Source materials

##### 2.1.1 Strains of bacteria

Strains of phase I *B. pertussis* shall be used.

##### 2.1.2 Culture medium

The medium used for cultivation of bacteria shall be free from human blood substances. When the medium containing animal blood has been used, any blood components must be removed by an appropriate method.

#### 2.2 Bulk material

A culture of incubated bacteria shall be tested for bacterial purity by microscopic examination and by inoculation into an appropriate medium. The culture confirmed to be free from any contaminant microorganisms shall be used, and a protective antigen fraction shall be prepared and purified by physicochemical procedures such as ammonium-sulfate fractionation, sucrose density gradient centrifugation. After purification, the protective antigen fraction shall be treated with formaldehyde or other appropriate method to inactivate the toxic activity. The liquid preparation containing purified detoxified protective antigen(s) shall serve as the bulk material.

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

#### 2.3 Final bulk

The bulk material shall be diluted with buffered physiological saline, supplemented with aluminum salt, and mixed to meet the potency required in 3.2.12. The pertussis antigen content shall be not more than 20 µg/mL. Appropriate preservatives and stabilizer may be added.

### 3. Control tests

#### 3.1 Tests on bulk material

##### 3.1.1 Staining test

The test given in General Tests shall apply.

##### 3.1.2 Sterility test

The sterility test given in General Tests shall apply.

##### 3.1.3 Inactivation test

When the test sample is cultured on blood agar medium or other suitable medium, no growth of bacteria shall be observed.

##### 3.1.4 Test for freedom from heat-labile toxin

The test sample shall be prepared in a concentration not less than two-fold that of the final bulk. The test sample shall be diluted, if necessary, with physiological saline.

The diluted test sample shall be given by intracutaneous injection at a dose of 0.025 mL into at least 4 suckling mice aged 48–72 hours, or into at least 2 rabbits weighing 2.0 to 4.0 kg at a dose of 0.1 mL. The inoculated animals shall be observed for 4 days. No animal shall show any local lesion

due to the heat-labile toxin of bacteria.

#### 3.1.5 Bacterial endotoxins test

The test sample shall be prepared in a concentration equal to that of the final bulk. The test sample shall be diluted, if necessary, with distilled water prepared for exclusive use in bacterial endotoxins test. When the test given in General Tests is applied, the endotoxin content shall be no higher than 2.0 EU/mL.

#### 3.1.6 Test for mouse leukocytosis-promoting toxicity

The test sample shall be prepared in a concentration equal to that of the final bulk. The test sample shall be diluted, if necessary, with physiological saline.

The test given in 3.2.10 shall apply.

#### 3.1.7 Test for mouse histamine-sensitizing toxicity

The test sample shall be prepared in a concentration equal to that of the final bulk. The test sample shall be diluted, if necessary, with physiological saline. The test given in 3.2.11 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 5.4 to 7.4.

#### 3.2.2 Test for aluminum content

When the test given in General Tests is applied, the aluminum content shall be no higher than 0.3 mg/mL.

#### 3.2.3 Test for thimerosal content

When thimerosal has been added as a preservative, the test given in General Tests shall apply. The thimerosal content shall be no higher than 0.012 w/v%.

#### 3.2.4 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%.

#### 3.2.5 Sterility test

The sterility test given in General Tests shall apply.

#### 3.2.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.2.7 Bacterial endotoxins test

When the test given in General Tests is applied, the endotoxin content shall be no higher than 4.0 EU/mL.

#### 3.2.8 Protein content

When the test for protein content given in General Tests is applied, the protein content in the test sample shall be no higher than 20 µg/mL.

#### 3.2.9 Test for mouse body weight-decreasing toxicity

##### 3.2.9.1 Materials

The test sample and Reference Pertussis Vaccine (for toxicity test) (hereafter referred to as "Toxicity Reference" in this monograph) shall be used.

##### 3.2.9.2 Test procedures

Toxicity Reference shall be diluted to make suitable logarithmic serial dilutions. The test sample and each dilution of Toxicity Reference shall be given by intraperitoneal injection into at least 10 mice aged 4 weeks at a dose of 0.5 mL. The body weight shall be recorded about 16 hours after injection and the difference with the weight before injection shall be calculated.

#### 3.2.9.3 Criterion for judgment

The body weight-decreasing toxicity of the test sample shall be no greater than 10 BWDU/mL in mice upon statistical analysis.

### 3.2.10 Test for mouse leukocytosis-promoting toxicity

#### 3.2.10.1 Materials

The test sample and Toxicity Reference shall be used.

#### 3.2.10.2 Test procedures

Toxicity Reference shall be diluted to make suitable logarithmic serial dilutions. The test sample and each dilution of Toxicity Reference shall be given by intraperitoneal injection into at least 10 mice aged 4 weeks at a dose of 0.5 mL. Leukocytes present in peripheral blood shall be counted 3 days after injection.

#### 3.2.10.3 Criterion for judgment

The leukocytosis-promoting toxicity of the test sample shall be no greater than 0.5 LPU/mL in mice upon statistical analysis.

### 3.2.11 Test for histamine-sensitizing toxicity

#### 3.2.11.1 Materials

The test sample, Toxicity Reference and histamine-dihydrochloride shall be used. The test shall be conducted in mice using two test samples, one of which has been kept at 37°C for 4 weeks and the other not warmed. The Toxicity Reference and histamine-dihydrochloride shall be diluted with physiological saline.

#### 3.2.11.2 Test procedures

Toxicity Reference shall be diluted to make suitable logarithmic serial dilutions. The test sample and each dilution of Toxicity Reference shall be given by intraperitoneal injection into at least 10 mice aged 4 weeks at a dose of 0.5 mL. Four days after injection, 4 mg of histamine-dihydrochloride shall be inoculated into each mouse. The rectal temperature shall be recorded 30 minutes after histamine injection.

#### 3.2.11.3 Criterion for judgment

The histamine-sensitizing toxicity of both test samples with or without warming at 37°C shall be no higher than 0.4 HSU/mL in mice upon statistical analysis.

### 3.2.12 Potency test

Potency shall be determined in mice by the intracerebral challenge method.

#### 3.2.12.1 Materials

The test sample, Standard Pertussis Vaccine (hereafter referred to as "Standard" in this monograph) and strain 18323 of *Bordetella pertussis* for challenge (hereafter referred to as "challenge strain" in this monograph) shall be used. The diluent for the test sample and Standard shall be sterile physiological saline.

The challenge strain shall be cultured on Bordet-Gengue blood agar medium for approximately

24 hours and suspended in 1 w/v% casamino acid solution containing 0.6 w/v% of sodium chloride (pH 7.0–7.2) to a concentration of approximately 200 LD<sub>50</sub> per 0.025 mL to serve as the suspension for challenge.

#### 3.2.12.2 Test procedures

The test sample and Standard shall be diluted serially to make at least three levels of four-fold or other suitable logarithmic dilutions. Each dilution shall be given by intraperitoneal injection at a dose of 0.5 mL into at least 16 mice aged 4 weeks. The animals shall be of the same sex or both sexes in the equal numbers. The challenge suspension shall be given by intracerebral injection into the animals at a dose of 0.025 mL 21 days after immunizing injection. The animals shall be observed for 14 days. Any animals dying within 3 days after challenge shall be excluded from the test. Any animals showing paralysis or swelling of the head at the end of the observation period shall be included in deaths.

At least 3 appropriate serial dilutions of the challenge suspension shall be injected into at least 10 mice aged 7 weeks to titrate the virulence. The bacterial count for the LD<sub>50</sub> per 0.025 mL of the challenge suspension shall be 50–400 cells. The live cell count in the challenge suspension obtained by culturing on Bordet-Gengue blood agar medium shall be approximately one-fourth of the total bacterial count obtained by the opacity test given in General Tests. The optical density of the test material containing  $1.0 \times 10^{10}$  of bacteria per mL shall correspond to 10 opacity units.

#### 3.2.12.3 Criterion for judgment

The potency of the test sample shall be no less than 8 U/mL upon statistical analysis.

#### 3.2.13 Identity test

Agar gel immunodiffusion test or other suitable antigen-antibody reaction method shall be applied for the test sample solubilized with sodium citrate or other appropriate reagent.

### **4. Storage and expiry date**

The expiry date shall be two years.

### **5. Other requirements**

#### 5.1 Information to be provided in package insert and other labeling

- (1) Names of strains used for production
- (2) Caution that the product be rendered homogenous by thorough shaking before use
- (3) Recommended human dose and route of administration, as follows:

For primary immunization, usually 3 doses of 0.5 mL are given by subcutaneous injection at intervals of 3–8 weeks.

For booster immunization, usually 0.5 mL is given by subcutaneous injection with an interval of not shorter than 6 months after completion of the primary immunization, (in general, during 12 and 18 months after completion of the primary immunization).

## DIPHTHERIA-PERTUSSIS COMBINED VACCINE

### 1. Descriptive definition

“Diphtheria-Pertussis Combined Vaccine” is a whitish turbid liquid product containing inactivated *Bordetella pertussis* (hereafter referred to as "bacteria" in this monograph) and "diphtheria toxoid".

### 2. Production control

#### 2.1 Source materials

Requirements given in 2.1 of Pertussis Vaccine and 2.1 of Diphtheria Toxoid shall apply.

#### 2.2 Bulk material

Requirements given in 2.2 of Pertussis Vaccine and 2.2 of Diphtheria Toxoid shall apply.

#### 2.3 Final bulk

The bulk material shall be diluted with buffered physiological saline or other suitable medium and mixed to render the bacterial content not higher than  $2.0 \times 10^{10}$  cells/mL when determined by the test given in 3.1.2 of Pertussis Vaccine. The content of diphtheria toxoid shall be no higher than 50 Lf/mL to serve as the final bulk.

Appropriate preservatives and stabilizer may be added.

The final bulk shall be subjected to the tests given in 3.2.

### 3. Control tests

#### 3.1 Tests on bulk material

Requirements given in 3.1 of Pertussis Vaccine and 3.1 of Diphtheria Toxoid shall apply.

#### 3.2 Tests on final bulk

##### 3.2.1 Test for thimerosal content

When thimerosal has been added as a preservative, the test given in General Tests shall apply. The thimerosal content shall be no higher than 0.012 w/v%.

##### 3.2.2 Staining test

The test given in General Tests shall apply.

##### 3.2.3 Sterility test

The test given in General Tests shall apply.

##### 3.2.4 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

##### 3.2.5 Test for toxicity to mouse weight gain

Requirements given in 3.3.8 of Pertussis Vaccine shall apply.

#### 3.3 Tests on final product

Following tests shall apply to each final lot.

##### 3.3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

#### 3.3.2 Test for protein nitrogen content

When the test given in General Tests is applied, the protein nitrogen content shall be no higher than 150 µg/mL.

#### 3.3.3 Test for thimerosal content

When thimerosal has been added as a preservative, the test given in General Tests shall apply. The thimerosal content shall be no higher than 0.012 w/v%.

#### 3.3.4 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%.

#### 3.3.5 Test for bacterial content

Requirements given in 3.3.5 of Pertussis Vaccine shall apply.

#### 3.3.6 Sterility test

The sterility test given in General Tests shall apply.

#### 3.3.7 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.3.8 Test for toxicity to mouse weight gain

Requirements given in 3.3.8 of Pertussis Vaccine shall apply.

#### 3.3.9 Test for mouse leukocytosis-promoting toxicity

Requirements given in 3.3.9 of Pertussis Vaccine shall apply.

#### 3.3.10 Test for detoxification of diphtheria toxin

Requirements given in 3.2.6.2 of Diphtheria Toxoid shall apply, excluding the test on the sample kept at 37°C for 20 days.

#### 3.3.11 Potency test

Requirements given in 3.3.10 of Pertussis Vaccine and those in 3.2.7 of Diphtheria Toxoid shall apply; however, the Standard Diphtheria Toxoid listed in 3.2.7.1.1 shall read Reference Diphtheria Toxoid for Combined Vaccine use. The diluent of the test sample and the Reference shall be physiological saline.

#### 3.3.12 Identity test

Requirements given in 3.3.11 of Pertussis Vaccine and 3.2.8 of Diphtheria Toxoid shall apply. To apply 3.2.8 of Diphtheria Toxoid, the test shall be conducted on the centrifugal supernatant of the test sample.

### **4. Storage and expiry date**

The expiry date shall be 18 months.

### **5. Other requirements**

#### 5.1 Information to be provided in package insert and other labeling

- (1) Names of strains used for production

- (2) The method used for inactivation
- (3) Caution that the product be rendered homogenous by thorough shaking before use
- (4) Recommended human dose and route of administration, as follows:

For primary immunization, usually 3 doses of 0.5 mL are given by subcutaneous injection at intervals of 3–8 weeks.

For booster immunization, usually 0.5 mL is given by subcutaneous injection with an interval of no less than 6 months after completion of the primary immunization, (in general, between 12 to 18 months after completion of the primary immunization).

## DIPHTHERIA-PERTUSSIS-TETANUS COMBINED VACCINE

### 1. Descriptive definition

“Diphtheria-Pertussis-Tetanus Combined Vaccine” is a whitish turbid liquid product containing inactivated *Bordetella pertussis* (hereafter referred to as "bacteria" in this monograph), "diphtheria toxoid" and "tetanus toxoid."

### 2. Production control

#### 2.1 Source materials

Requirements given in 2.1 of Pertussis Vaccine, 2.1 of Diphtheria Toxoid and 2.1 of Tetanus Toxoid shall apply.

#### 2.2 Bulk material

Requirements given in 2.2 of Pertussis Vaccine, 2.2 of Diphtheria Toxoid and 2.2 of Tetanus Toxoid shall apply.

#### 2.3 Final bulk

The bulk material shall be diluted with buffered physiological saline or other suitable medium and mixed to render the bacterial content no higher than  $2.0 \times 10^{10}$  cells/mL when determined by the test given in 3.1.2 of Pertussis Vaccine. The content of diphtheria toxoid shall be no higher than 50 Lf/mL and the content of tetanus Toxoid shall be no higher than 12.5 Lf/mL to serve as the final bulk.

Appropriate preservatives may be added.

The final bulk shall be subjected to the tests given in 3.2.

### 3. Control tests

#### 3.1 Tests on bulk material

Requirements given in 3.1 of Pertussis Vaccine, 3.1 of Diphtheria Toxoid and 3.1 of Tetanus Toxoid shall apply.

#### 3.2 Tests on final bulk

##### 3.2.1 Test for thimerosal content

When thimerosal has been added as a preservative, the test given in General Tests shall apply. The thimerosal content shall be no higher than 0.012 w/v%.

##### 3.2.2 Staining test

The test given in General Tests shall apply.

##### 3.2.3 Sterility test

The test given in General Tests shall apply.

##### 3.2.4 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

##### 3.2.5 Test for toxicity to mouse weight gain

Requirements given in 3.3.8 of Pertussis Vaccine shall apply.

### 3.3 Tests on final product

Following tests shall apply to each final lot.

#### 3.3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

#### 3.3.2 Test for protein nitrogen content

When the test given in General Tests is applied, the protein nitrogen content shall be no higher than 150 µg/mL.

#### 3.3.3 Test for thimerosal content

When thimerosal has been added as a preservative, the test given in General Tests shall apply. The thimerosal content shall be no higher than 0.012 w/v%

#### 3.3.4 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%.

#### 3.3.5 Test for bacterial content

Requirements given in 3.3.5 of Pertussis Vaccine shall apply.

#### 3.3.6 Sterility test

The test given in General Tests shall apply.

#### 3.3.7 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.3.8 Test for toxicity to mouse weight gain

Requirements given in 3.3.8 of Pertussis Vaccine shall apply.

#### 3.3.9 Test for mouse leukocytosis-promoting toxicity

Requirements given in 3.3.9 of Pertussis Vaccine shall apply.

#### 3.3.10 Test for detoxification of diphtheria toxin

Requirements given in 3.2.6.2 of Diphtheria Toxoid shall apply, excluding the test on samples kept at 37°C for 20 days.

#### 3.3.11 Test for detoxification of tetanus toxin

The test sample shall be given by subcutaneous injection at a dose of 3 mL into at least 4 guinea pigs weighing 300–400 g. The animals shall be observed for at least 21 days. No animal shall die due to intoxication, or show any of such specific symptoms of intoxication as spasms, stiffening, pronounced decrease in body weight or other abnormal sign during the observation period.

#### 3.3.12 Potency test

Requirements given in 3.3.10 of Pertussis Vaccine, 3.2.7 of Diphtheria Toxoid and 3.2.7 of Tetanus Toxoid shall apply. However, the Standard Toxioids for Diphtheria and Tetanus Toxioids in each description of 3.2.7.1.1 of Diphtheria and Tetanus Toxioids shall read Reference Diphtheria and Tetanus Toxoid for Combined Vaccine use, respectively. The diluent of the test sample and the Reference shall be physiological saline.

#### 3.3.13 Identity test

Requirements given in 3.3.11 of Pertussis Vaccine, 3.2.8 of Tetanus Toxoid and 3.2.8 of

Diphtheria Toxoid shall apply. To apply 3.2.8 of Diphtheria Toxoid and 3.2.8 of Tetanus Toxoid, the test shall be conducted on the centrifugal supernatant of the test sample.

**4. Storage and expiry date**

The expiry date shall be 18 months.

**5. Other requirements**

5.1 Information to be provided in package insert and other labeling

- (1) Names of strains used for production
- (2) Method used for the inactivation of bacteria
- (3) Caution that the product be rendered homogenous by thorough shaking before use
- (4) Recommended human dose and route of administration, as follows:

For primary immunization, usually 3 doses of 0.5 mL are given by subcutaneous injection with intervals of 3–8 weeks.

For booster immunization, usually 0.5 mL is given by subcutaneous injection with an interval of not less than 6 months after completion of the primary immunization, (in general, between 12 to 18 months after completion of the primary immunization).

# ADSORBED DIPHTHERIA-PURIFIED PERTUSSIS-TETANUS COMBINED VACCINE

## 1. Descriptive definition

“Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine” is a liquid product containing protective antigen(s) of *Bordetella pertussis*, "diphtheria toxoid" and "tetanus toxoid," and rendered insoluble by adding aluminum salt. When shaken well, it becomes a whitish turbid homogeneous liquid.

## 2. Production control

### 2.1 Source materials

Requirements given in 2.1 of Adsorbed-Purified Pertussis Vaccine, 2.1 of Diphtheria Toxoid and 2.1 of Tetanus Toxoid shall apply.

### 2.2 Bulk material

Requirements given in 2.2 of Adsorbed-Purified Pertussis Vaccine, 2.2 of Diphtheria Toxoid and 2.2 of Tetanus Toxoid shall apply.

### 2.3 Final bulk

The bulk material shall be diluted with buffered physiological saline or other suitable medium and requirements given in 2.3 of Adsorbed-Purified Pertussis Vaccine and 2.3 of Adsorbed Diphtheria-Tetanus Combined Toxoid shall apply.

Appropriate preservatives and stabilizer may be added.

## 3. Control tests

### 3.1 Tests on bulk material

Requirements given in 3.1 of Adsorbed-Purified Pertussis Vaccine, 3.1 of Diphtheria Toxoid and 3.1 of Tetanus Toxoid 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 5.4 to 7.4.

#### 3.2.2 Test for aluminum content

When the test given in General Tests is applied, the aluminum content shall be no higher than 0.3mg/mL.

#### 3.2.3 Test for thimerosal content

When thimerosal has been added as a preservative and the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

#### 3.2.4 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%.

### 3.2.5 Sterility test

The test given in General Tests shall apply.

### 3.2.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.2.7 The bacterial endotoxins test

Requirements given in 3.2.7 of Adsorbed Purified Pertussis Vaccine shall apply. The endotoxin content shall be no higher than 4.0 EU/mL.

### 3.2.8 Test for mouse body weight-decreasing toxicity

Requirements given in 3.2.9 of Adsorbed Purified Pertussis Vaccine shall apply.

### 3.2.9 Test for mouse leukocytosis-promoting toxicity

Requirements given in 3.2.10 of Adsorbed Purified Pertussis Vaccine shall apply.

### 3.2.10 Test for mouse histamine-sensitizing toxicity

Requirements given in 3.2.11 of Adsorbed Purified Pertussis Vaccine shall apply.

### 3.2.11 Test for detoxification of diphtheria toxin

Requirements given in 3.2.6.2 of Diphtheria Toxoid shall apply, excluding the test on the sample kept at 37°C for 20 days.

### 3.2.12 Test for detoxification of tetanus toxin

The test sample shall be given by subcutaneous injection at a dose of 3 mL into at least 4 guinea pigs weighing 300–400 g. The animals shall be observed for at least 21 days. No animal shall die due to intoxication, or show any of specific symptoms of intoxication such as spasms, stiffening, pronounced decrease in body weight or other abnormal sign during the observation period.

### 3.2.13 Potency test

#### 3.2.13.1 Potency test for adsorbed purified pertussis vaccine

Potency shall be determined in mice by the intracerebral challenge method.

##### 3.2.13.1.1 Materials

The test sample, Standard Pertussis Vaccine (hereafter referred to as "Standard" in this monograph) and strain 18323 of *Bordetella pertussis* for challenge (hereafter referred to as "challenge strain" in this monograph) shall be used. The diluent for the test sample and Standard shall be sterile physiological saline. The challenge strain shall be cultured on blood agar medium for about 24 hours and suspended in casamino acid solution (1 w/v% casamino acids, 0.6 w/v% sodium chloride, pH 7.0–7.2) to a concentration of about 200 LD<sub>50</sub> per 0.025 mL to serve as the suspension for challenge.

##### 3.2.13.1.2 Test procedures

The test sample and Standard shall be diluted serially to make at least three levels of four-fold or other suitable logarithmic dilutions. Each dilution shall be given by intraperitoneal injection at a dose of 0.5 mL into at least 16 mice aged 4 weeks. The animals shall be of the same sex or both sexes in the equal numbers. The challenge suspension shall be given by intracerebral injection into the animals at a dose of 0.025 mL 21 days after immunizing injection. The animals shall be observed for 14 days. Any animals dying within 3 days after challenge shall be excluded from the test. Any animals showing paralysis or swelling of the head at the end of the observation period shall be included in deaths.

At least three appropriate serial dilutions of the challenge suspension shall be injected into at least 10 mice aged 7 weeks to titrate the virulence. The bacterial count for the LD<sub>50</sub> per 0.025 mL of the challenge suspension shall be 50–400 cells. The live cell count in the challenge suspension obtained by culturing on blood agar medium shall be approximately one-fourth of the total bacterial count obtained by the opacity test given in General Tests. The optical density of the test material containing  $1.0 \times 10^{10}$  of bacteria per mL shall correspond to 10 opacity units.

#### 3.2.13.1.3 Criterion for judgment

The potency of the test sample shall be no less than 8 U/mL upon statistical analysis.

#### 3.2.13.2 Potency test for Adsorbed Diphtheria Toxoid

Potency shall be determined by the diphtheria toxin challenge method or by the antitoxin titration method in guinea pigs or by the antitoxin titration method in mice.

##### 3.2.13.2.1 Toxin challenge method

###### 3.2.13.2.1.1 Materials

The test sample, Reference Adsorbed Diphtheria Toxoid for Combined Vaccine (hereafter referred to as "Reference" in this monograph) and an appropriate toxin solution shall be used. The test sample and Reference shall be diluted in physiological saline and toxin in 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

###### 3.2.13.2.1.2 Test procedures

The test sample and the Reference shall be diluted serially at equal logarithmic intervals. Each dilution shall be given by subcutaneous injection at a dose of 2 mL into at least 10 guinea pigs weighing 300–400 g.

The animals shall be challenged with approximately 50 LD<sub>50</sub> of toxin 4–6 weeks after immunizing injection. The animals shall be observed for 7 days.

The toxin used for challenge shall be titrated by injecting with at least three serial dilutions into at least 3 guinea pigs weighing 400–600 g. The challenge toxin shall contain 25–100 LD<sub>50</sub> per inoculum.

###### 3.2.13.2.1.3 Criterion for judgment

The potency of the test sample shall be no less than 47 U/mL upon statistical analysis.

##### 3.2.13.2.2 Antitoxin titration method

Antitoxin content of the serum shall be titrated by the rabbit intracutaneous method, cell culture method, or passive hemagglutination method.

###### 3.2.13.2.2.1 Materials

The test sample, the Reference, Standard Diphtheria Antitoxin and a toxin solution with a known binding capacity shall be used. When the passive hemagglutination method is used, red blood cells shall be sensitized with diphtheria toxin or toxoid of the purity of not less than 2,500 Lf/mg protein nitrogen.

###### 3.2.13.2.2.2 Test procedures

Immunizing injection of animals shall be conducted by the method given in 3.2.13.2.1.2. Each dilution shall be given by subcutaneous injection at a dose of 0.5 mL into at least 10 mice aged 5 weeks when using mouse method.

Antitoxin content of the serum from each animal shall be determined 4 to 6 weeks after

immunization.

#### 3.2.13.2.2.3 Criterion for judgment.

The criterion provided in 3.2.13.2.1.3 shall apply.

#### 3.2.13.3 Potency test for Adsorbed Tetanus Toxoid

Potency shall be measured in guinea pigs or mice by the tetanus toxin challenge method or the antitoxin titration method.

##### 3.2.13.3.1 Toxin challenge method

###### 3.2.13.3.1.1 Materials

The test sample, Reference Adsorbed Tetanus Toxoid (for combined Vaccine) (hereafter referred to as "Reference" in this monograph) and an appropriate solution of toxin shall be used. The test sample and the Reference shall be diluted in physiological saline, and the toxin in 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

###### 3.2.13.3.1.2 Test procedures

The test sample and Reference shall be diluted serially at equal logarithmic intervals. Each dilution shall be given by subcutaneous injection into at least 10 guinea pigs weighing 300–400 g at a dose of 2 mL or at least 10 mice aged 5 weeks at a dose of 0.5mL. The guinea pigs or mice shall be challenged with approximately 50 or 100 LD<sub>50</sub> of toxin, respectively, 4–6 weeks after immunizing injection. The challenged animals shall be observed for 4 days.

The toxin used for challenge shall be titrated by injecting at least three serial dilutions into at least 3 guinea pigs each weighing 400–600 g or at least 3 mice aged 4 weeks. The challenge toxin shall contain 25 to 100 LD<sub>50</sub> per inoculum for guinea pigs or 50 to 200 LD<sub>50</sub> per inoculum for mice.

###### 3.2.13.3.1.3 Criterion for judgment

The potency of the test sample shall be no less than 27 U/mL upon statistical analysis.

##### 3.2.13.3.2 Antitoxin titration method

###### 3.2.13.3.2.1 Materials

The test sample, Reference and a toxin solution with known binding capacity shall be used. Dilutions of these materials shall be made according to 3.2.13.3.1.1

###### 3.2.13.3.2.2 Test procedures

Immunization of animals shall be conducted by the method given in 3.2.13.3.1.2. Antitoxin content shall be determined by the mouse method on the serum from each animal 4 to 6 weeks after immunization.

###### 3.2.13.3.2.3 Criterion for judgment

The criterion provided in 3.2.13.3.1.3 shall apply.

#### 3.2.14 Identity test

Requirements given in 3.2.13 of Precipitated-Purified Pertussis Vaccine, 3.2.9 of Adsorbed Diphtheria Toxoid and 3.2.9 of Adsorbed Tetanus Toxoid shall apply for the test sample solubilized with sodium citrate or other appropriate medium.

## 4. Storage and expiry date

The expiry date shall be two years.

## **5. Other requirements**

### 5.1 Information to be provided in package insert and other labeling

- (1) Names of strains used for production
- (2) Caution that the product be rendered homogenous by thorough shaking before use
- (3) Recommended human dose and route of administration, as follows:

For primary immunization, usually 3 doses of 0.5 mL are given by subcutaneous injection at intervals of 3–8 weeks. For booster immunization, usually 0.5 mL is given by subcutaneous injection at an interval of not less than 6 months after completion of the primary immunization, (in general, 12–18 months after completion of the primary immunization).

# **FREEZE-DRIED LIVE ATTENUATED RUBELLA VACCINE**

## **1. Descriptive definition**

“Freeze-dried Live Attenuated Rubella Vaccine” is a freeze-dried product containing attenuated rubella virus (hereafter referred to as "virus" in this monograph). When reconstituted, it becomes a colorless or reddish clear liquid.

## **2. Production control**

### 2.1 Source materials

#### 2.1.1 Strains of virus used for production

Strains of virus approved as suitable for production shall be used. Virus contained in the product shall be passaged under the approved culture conditions and shall not be passaged more than 5 times from the original vaccine approved as adequate for the strain for production.

#### 2.1.2 Animals and embryonated eggs used for production

Rabbit renal tissue cultures used for propagation of virus shall be obtained from healthy rabbits only. Each rabbit shall be observed during the quarantine period of no less than 7 days for the presence of signs or symptoms of ill health as well as fever. At the time of autopsy animals shall be examined for evidence of salmonellosis, tuberculosis, pseudotuberculosis, myxoedema or other diseases indigenous to rabbits. If any such signs, symptoms or other significant pathological lesions which would render the animal unsuitable for vaccine production are observed, tissues from that animal shall not be used. Embryonated quail eggs used as a source of quail embryo tissue for the propagation of virus shall be derived from flocks certified to be free from infectious diseases.

#### 2.1.3 Culture media

The cell culture medium can be supplemented with appropriate cell growth-promoting substances, phenol red at a concentration not higher than 0.002 w/v%, and minimal amounts of antibiotics, except penicillin. If non-human serum or fractions thereof are added to the medium, serum albumin concentration in the final bulk shall be controlled to be no higher than 50 ng per dose. The culture medium used for the propagation of virus can be supplemented with phenol red at a concentration not higher than 0.002 w/v%, appropriate stabilizer and minimal amounts of antibiotics. However, no non-human serum or fractions thereof, nor penicillin shall be added.

### 2.2 Bulk material

#### 2.2.1 Cell culture

Cells from individual animals shall be cultured separately.

Prior to the inoculation of virus, no cytopathic change shall be detected in any cell culture. Rabbit kidney cell cultures obtained from a litter of rabbits may be regarded as an "individual cell culture". Quail embryonic cell cultures obtained in one session shall be regarded as an "individual cell culture". The individual cell cultures shall be subjected to the tests given in 3.1.

#### 2.2.2 Virus suspension

The virus suspension harvested from individual cell cultures shall be pooled to serve as a "single harvest". The single harvest shall be subjected to the tests given in 3.2.1.

The single harvest shall be pooled to make a virus suspension before filtration. Appropriate

stabilizer may be added to the virus suspension before filtration. The virus suspension before filtration shall be subjected to the tests given in 3.2.2.

### 2.2.3 Filtration

The virus suspension before filtration shall be centrifuged and/or filtrated to eliminate cells, and the suspension shall be combined to make the bulk material. The bulk material shall be tested as directed in 3.3.

### 2.3 Final bulk and freeze-drying

The bulk material shall be diluted, if required, to make the final bulk. Appropriate stabilizer may be used. However, antibiotics shall not be added. The final bulk shall be filled into final containers and freeze-dried. The final bulk shall be tested as directed in 3.4.

## 3. Control tests

### 3.1 Tests on individual cell cultures

A volume equivalent to 25% or 500 mL of individual cell cultures shall be used as the control culture of uninfected cells. The control cell culture shall be subjected to the tests given below.

#### 3.1.1 Observation of cell culture

The control cell cultures shall be cultivated without being inoculated with the virus under the same conditions used for virus infected cell cultures. No cytopathic change due to extraneous virus(s) shall be detected. During the observation period, control cell cultures discarded due to any nonspecific or incidental reason shall be less than 20% of the total control cells cultures.

#### 3.1.12 Tests in cell cultures

At the end of the above observation period, the culture medium shall be harvested from the vessel, pooled if necessary, and be tested as directed in 3.3.3.2

### 3.2 Tests on virus suspension

#### 3.2.1 Tests on single harvest

##### 3.2.1.1 Sterility test

Sterility Test and Mycoplasma Test given in General Tests shall apply.

##### 3.2.1.2 Test for freedom from extraneous viruses

The test given in 3.3.3.2 shall apply. The test shall be performed, if necessary, after the neutralization of virus with ant-rubella serum. For neutralization of virus derived from rabbit kidney and quail embryo cells, antiserum prepared in animals except humans, monkeys and rabbits and that prepared in animals except humans, monkeys, adult chickens and quails, respectively, shall be used.

#### 3.2.2 Tests on virus suspension before filtration

##### 3.2.2.1 Sterility test

Sterility Test, Mycoplasma Test, Mycobacterial Sterility Test given in General Tests shall apply. For the Mycobacterial Sterility Test, a 25-mL portion of the test sample shall be centrifuged and resuspended in 5 mL of physiological saline prior to use.

### 3.3 Tests on bulk material

In the following specifications, "bulk of rabbit origin" denotes bulk material prepared from virus suspensions derived from rabbit kidney cell cultures and "bulk of quail origin" denotes that

prepared from quail embryo cell cultures, respectively.

The bulk material shall be subjected to the tests given below after being diluted to the same concentration as the final bulk.

#### 3.3.1 Staining test

The test given in General Tests shall apply.

#### 3.3.2 Sterility test

The test given in General Tests shall apply.

#### 3.3.3 Test for freedom from extraneous viruses

The test shall be performed after neutralization of rubella virus, if necessary, according to the procedures given in 3.2.1.2.

##### 3.3.3.1 Tests in animals

###### 3.3.3.1.1 Inoculation of adult mice

At least 10 mice aged 4–5 weeks shall be inoculated intraperitoneally with 0.5 mL and intracerebrally with 0.03 mL of the sample. The mice shall be observed for 21 days. No animal shall show evidence of infection with extraneous microbial agents, and more than 80% of the inoculated mice must survive the observation period.

###### 3.3.3.1.2 Inoculation of suckling mice

Within 24 hours after birth, suckling mice shall be inoculated intraperitoneally with 0.1 mL and intracerebrally with 0.01 mL of the test sample. The mice shall be observed for 14 days. Except those died within the first 24 hours of the test, no mouse of at least 20 mice shall show evidence of infection with extraneous microbial agents, and more than 80% of the mice must survive the observation period.

###### 3.3.3.1.3 Intracerebral inoculation of guinea pigs

At least 5 guinea pigs of 300–400 g weight shall be inoculated intracerebrally with 0.1 mL of the test sample. The guinea pigs shall be observed for 14 days. No guinea pig shall show evidence of infection with extraneous microbial agents, and more than 80% of the guinea pigs must survive the observation period.

###### 3.3.3.1.4 Inoculation of rabbits

The test shall be performed on the bulk of rabbit origin only. At least 5 rabbits weighing 1.5–2.5 kg shall be inoculated intradermally in multiple sites with a total of 1.0 mL each and subcutaneously with 9.0 mL each of the test sample. The animals shall be observed for 35 days. No animal shall show evidence of infection with extraneous virus and more than 80% of the animals must survive the observation period.

##### 3.3.3.2 Tests in cell cultures

###### 3.3.3.2.1 Inoculation of human tissue culture cells

A 10-mL portion of the test sample shall be inoculated into human cells. The inoculated cells shall be cultured for 7 days, and portions of the cells shall be passaged by culture for a further 7 days. No cytopathic change due to extraneous viruses shall be detected within the entire culture period.

###### 3.3.3.2.2 Inoculation of rabbit renal tissue cell cultures

The test shall be performed on the bulk of rabbit origin only. A 10-mL portion of the test sample

shall be inoculated into primary cultures of rabbit kidney cells. The cells shall be incubated and observed for 14 days. On the 14th day of incubation, the cell culture shall be frozen and thawed, and subsequently inoculated into a fresh culture of primary rabbit kidney cells. The inoculated cells shall be incubated and observed for 14 days. At the end of the observation period, the cells shall be incubated with red blood cell suspensions originated from guinea pig and adult chicken, respectively. No hemadsorption shall be observed. During the observation period, no cytopathic change due to extraneous viruses shall be detected.

#### 3.3.3.2.3 Inoculation of primary tissue cultures of embryonic chick cells

The test shall be performed on the bulk of quail origin only. A 25-mL portion of the test sample shall be inoculated into primary cultures of chick embryo cells. The inoculated cells shall be cultured and passaged 3 times. No avian leukemia viruses shall be detected in the cultured cells or fluid using an approved method such as enzyme-linked immunosolvent assay. In addition, no reticuloendotheliosis virus antigen shall be stained immunologically in the cultured cells using the anti-reticuloendotheliosis virus serum

#### 3.3.3.2.4 Inoculation of primary tissue cultures of embryonic quail cells

The test shall be performed on the bulk of quail origin only.

A 5-mL portion of the test sample shall be inoculated into primary tissue cultures of embryonic quail cells. The inoculated cells shall be incubated and observed for 14 days. On the 14th day of incubation, the cell cultures shall be frozen and thawed, and subsequently inoculated into a fresh primary culture of embryonic quail cells. The cells shall be incubated and observed for 14 days. At the end of the observation period, the cells shall be incubated with red blood cell suspensions originated from guinea pig and adult chicken, respectively. No hemadsorption shall be observed. During the observation period, no cytopathic change due to extraneous viruses shall be detected.

#### 3.3.3.2.5 Inoculation of primary renal tissue cultures of chick cells

The test shall be performed on the bulk of quail origin only. A 5-mL portion of the test sample shall be inoculated into primary cultures of chick kidney cells. Inoculated cells shall be incubated and observed for 14 days. Then cells shall be frozen and thawed to induce passage, and subsequently inoculated into fresh primary cultures of chick kidney cells. At the end of the observation period, the cells shall be incubated with red blood cell suspensions originated from guinea pig and adult chicken, respectively. No cell-hemadsorption shall be detected. No cytopathic change due to extraneous viruses shall be detected during any observation period.

#### 3.3.3.3 Inoculation of embryonated chicken eggs

The test shall be performed on the bulk of quail origin only. At least 20 embryonated chicken eggs aged 10–11 days each inoculated with 0.25 mL of test sample onto the chorioallantoic membranes or into the allantoic cavity shall be incubated for 3 days. At least 20 embryonated chicken eggs aged 6–7 days each inoculated with 0.25 mL of the test sample into the yolk sac shall be incubated for 12 days. No egg, except those died within the first 24 hours of the test, shall show evidence of infection with extraneous viruses, and more than 80% of the eggs must survive the observation period. Samples obtained from eggs that died after the first 24 hours of the test shall be reinoculated into at least 10 additional eggs by the same inoculation route, and these should be incubated for the same number of days. No egg shall show evidence of infection with extraneous viruses, and more than 80% of the eggs must survive the observation period.

#### 3.3.4 Identification test

The propagation of virus in the sample shall be suppressed in appropriate cell cultures after neutralization with anti-rubella virus serum.

#### 3.3.5 Neurovirulence safety test

The test shall be performed in *Macacus* or *Cercopithecus* monkeys that have been shown to be serologically negative for rubella. At least 10 monkeys shall be employed in each test. The test sample shall be injected into the thalamic region of each hemisphere at a dose of 0.5 mL and into the cerebellomedullary cistern at a dose of 0.25 mL. The animals shall be observed for 21 days. No signs of paralysis or other neurological disorders shall be detected, and more than 80% of the animals must survive the observation period. No animal shall show abnormal clinical signs or death due to the virus or extraneous microbial agents. At the end of the observation period, each animal shall be autopsied for histopathological examination of the central nervous system. No evidence of changes due to the presence of unusual neurotropism by the virus or to the presence of extraneous microbial agents in test samples of central nervous system shall be demonstrated. In addition, animals that demonstrate the possibility of immunodeficiency as suggested by clinical signs and symptoms and histopathological findings, and those that show an underlying immunologic disease shall be excluded from the test. In consecutive tests of preparations from the same strain of the virus, no apparent differences between tests shall be demonstrated in any data from all of the animals used, including differences in the character, severity, and spread of lesions, and clinical signs during observation.

If each of the first 5 consecutive lots of bulk material prepared from a seed virus strain satisfies the requirements above, subsequent lots prepared from the same seed virus strain shall not be required to undergo the neurovirulence safety test.

#### 3.3.6 Marker test

At least 10 guinea pigs weighing about 300–400 g shall be inoculated subcutaneously with 1,000–10,000 PFU, FFU or CCID<sub>50</sub> each of the test sample. Thirty-five days after inoculation, blood samples shall be taken for serological tests. More than 80% of inoculated guinea pigs shall be antibodically negative for rubella.

#### 3.3.7 Test for virus content

The test given in 3.5.3 shall apply.

### 3.4 Tests on final bulk

#### 3.4.1 Staining test

The test given in General Tests shall apply.

#### 3.4.2 Sterility test

The test given in General Tests shall apply.

#### 3.4.3 Test for virus content

The final bulk shall be tested for rubella virus concentration according to the procedure given in 3.5.3

#### 3.4.4 Test for freedom from abnormal toxicity

The final bulk shall be tested for the absence of abnormal toxicity according to the procedure given in General Tests.

### 3.5 Tests on final product

Following tests shall apply to each final lot.

3.5.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the procedures given in General Tests.

3.5.2 Sterility test

The test given in General Tests.

3.5.3 Potency test

The concentration of mumps virus in the final product shall be determined by titration in a suitable cell culture system. The potency of the test sample shall be shown as PFU, FFU, or CCID<sub>50</sub>. The minimum acceptable virus titer per 0.5 mL shall be 1,000.

3.5.4 Identity test

Rubella virus in the final product shall be identified by immunofluorescence after propagation of virus in an appropriate cell culture.

**4. Storage and expiry date**

The final product shall be kept at 5 °C or less during storage. The expiry date shall be one year.

**5. Other requirements**

5.1 Reconstituent

The reconstituent shall be water for injection.

5.2 Information to be provided in package insert and other labeling

- (1) Name of the virus strain used for production.
- (2) Name of the cell culture used for the cultivation of virus.
- (3) Names and concentrations of antibiotics or dyes used in the cultivation of virus, if applicable.
- (4) Names and concentrations of stabilizer, if used.
- (5) The recommended human dosage and route of administration is as follows:

Usually, 0.5 mL is given by subcutaneous injection.

# TYPHUS VACCINE

## 1. Descriptive definition

“Typhus Vaccine” is a colorless to yellowish brown clear or slightly whitish turbid liquid product containing inactivated *Rickettsia prowazeki* (hereafter referred to as "rickettsia" in this monograph).

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Strain used for production

The Breinl strain of rickettsia shall be used.

#### 2.1.2 Developing hens eggs used for production

Hens eggs laid by healthy hens fed on feed containing no anti-rickettsial drugs and incubated for about 5–7 days (hereafter referred to as "egg" in this monograph) shall be used.

### 2.2 Bulk material

#### 2.2.1 Suspension of rickettsia

The strain for production shall be inoculated into the yolk sacs of eggs, and cultivated at about 35°C for 7–9 days. At the time when the embryos begin to die, the yolk sacs of live eggs shall be harvested, and triturated in physiological saline or other suitable medium to make an approximately 20 w/v% emulsion. The emulsion shall be centrifuged at approximately 800 × g, the supernatant shall be collected to serve as a suspension of rickettsia.

The rickettsia suspension shall be subjected to the tests given in 3.1.

#### 2.2.2 Inactivation and purification

Formaldehyde shall be added to the rickettsia suspension at a concentration of 0.2 w/v% which shall be held at approximately 25°C for 48–96 hours to inactivate rickettsia. After the completion of inactivation, the suspension shall be combined with twice the volume of ether, thoroughly mixed, and held at 5°C or less. The ether and turbid intermediate layers shall be discarded, and the bottom aqueous layer shall be collected. This treatment shall be repeated, if necessary, until the aqueous layer becomes almost clear.

After completion of the ether treatment, the aqueous layer shall be freed from ether under negative pressure at approximately 25°C. Thimerosal shall be added to the aqueous layer at a concentration of 0.01 w/v% to obtain the bulk material.

The bulk material shall be subjected to the tests given in 3.2.

### 2.3 Final bulk

The bulk material shall be diluted in buffered physiological saline or other suitable medium to render the concentration equivalent to 10 w/v% of the original yolk sac to serve as the final bulk. Appropriate preservatives and stabilizer may be added.

## 3. Control tests

### 3.1 Tests on suspension of rickettsia

#### 3.1.1 Staining test

The test given in General Tests with Giemsa's or other appropriate staining solution shall be applied to the sample without centrifugation.

### 3.2 Tests on bulk suspension

#### 3.2.1 Staining test

The test given in General Tests with Giemsa's staining solution shall apply.

#### 3.2.2 Sterility test

Sterility test and Mycoplasma test given in General Tests shall apply.

#### 3.2.3 Inactivation test

The test given in 3.4.6 shall apply, with at least 4 animals used.

The General Safety Test (the test for freedom of abnormal toxicity) given in General Tests shall apply.

### 3.3 Tests on final bulk

#### 3.3.1 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

#### 3.3.2 Sterility test

The test given in General Tests shall apply.

#### 3.3.3 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.4 Tests on final product

Following tests shall apply to each final lot.

#### 3.4.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

#### 3.4.2 Test for protein nitrogen content

When the test given in General Tests is applied, the protein nitrogen content shall be no higher than 800 µg/mL.

#### 3.4.3 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no higher than 0.012 w/v%.

#### 3.4.4 Test for formaldehyde content

When the test given in General Tests is applied, the formaldehyde content shall be no higher than 0.04 w/v%.

#### 3.4.5 Sterility test

The test given in General Tests shall apply.

#### 3.4.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.4.7 Inactivation test

The test sample shall be injected into at least 2 guinea pigs weighing 300–400 g. The animals shall be observed for 14 days. No animal shall show fever of 39.7°C or higher or other abnormal signs during the observation period.

#### 3.4.8 Potency test

Potency shall be determined in guinea pigs by the challenge method.

##### 3.4.8.1 Materials

The test sample and the strain of rickettsia for challenge (hereafter referred to as "challenge strain" in this monograph) shall be used. The challenge strain shall be given by intracerebral injection into guinea pigs weighing 300–400 g. The brains of the animals shall be harvested on the third day from the onset of symptoms of infection, and triturated in 0.013 mol/L phosphate-buffered sodium chloride solution (pH 7.0) into a 10 w/v% emulsion. The emulsion shall be centrifuged at approximately  $1,000 \times g$  for 5 minutes, and the supernatant shall serve as the suspension of rickettsia for challenge.

##### 3.4.8.2 Test procedures

The test sample shall be given by intraperitoneal injection into at least 8 guinea pigs weighing 300–400 g at 2 doses of 0.5 mL at an interval of 7 days. The rickettsia suspension for challenge shall be given by intraperitoneal injection into the animals at a dose of 1.0 mL 14 days after the second immunizing injection. The animals shall be observed for 14 days.

The rickettsia suspension for challenge shall be given by intraperitoneal injection into at least 8 nonimmune guinea pigs at a dose of 1.0 mL, and the animals shall be similarly observed. At least 80% of the nonimmune animals shall show symptoms of infection accompanied by fever of 39.7°C or higher during the observation period.

##### 3.4.8.3 Criterion for judgment

At least 80% of the immunized animals shall be free from symptoms of infection accompanied by fever of 39.7°C or higher.

#### 3.4.9 Identity test

The test shall be conducted by the serological method using anti-rickettsial immune serum.

### 4. Storage and expiry date

The expiry date shall be 18 months.

### 5. Other requirements

#### 5.1 Information to be provided in package insert and other labeling

- (1) Caution that the product be rendered homogenous by thorough shaking before use
- (2) Recommended human dose and route of administration, as follows:

For primary immunization, usually 2 doses of 1 mL are given by subcutaneous injection at an interval of 7–10 days. For booster immunization, usually 1 mL is given by subcutaneous injection within a period of 3 months after the completion of primary immunization.

## **FREEZE-DRIED BOTULISM ANTITOXIN, EQUINE**

### **1. Descriptive definition**

“Freeze-dried Botulism Antitoxin” is a freeze-dried product containing "botulism antitoxin type A," "botulism antitoxin type B," "botulism antitoxin type E" and "botulism antitoxin type F" in horse immunoglobulin (hereafter referred to as the respective "antitoxin" in this monograph). The product may contain 1 to 3 of the above 4 antitoxins. When reconstituted, it becomes a colorless or slightly yellowish brown, clear or slightly whitish turbid liquid.

### **2. Production control**

#### 2.1 Source materials

##### 2.1.1 Antigens used for immunization

*C. botulinum* toxins types A, B, E and F or corresponding toxoids shall be used for immunization.

##### 2.1.2 Animals used for production

Horses shall be used for the production of sera.

#### 2.2 Bulk material

##### 2.2.1 Crude antitoxic materials

Crude antitoxic serum or plasma shall be used if it contains no less than 300 units per mL with respect to type A, B and E, or 100 units per mL with respect to type F, and passes the sterility test and the pyrogen test given in General Tests.

##### 2.2.2 Purification

The fraction containing immunoglobulin shall be prepared by fractionating the crude antitoxic material using a suitable method that has been shown not to cause the deterioration of the antibodies. The fractions shall be treated with an appropriate proteolytic enzyme. The preparations containing the treated antitoxin shall serve as the bulk material.

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

#### 2.3 Final bulk and freeze-drying

The bulk material shall be diluted, if necessary, with buffered physiological saline or other suitable medium, mixed, dispensed and freeze-dried to make the final product. When reconstituted, 1mL of the product shall contain no fewer than 500 units of each antitoxin with respect to type A, B or E, and no fewer than 200 units with respect to type F.

### **3. Control tests**

#### 3.1 Tests on bulk material

##### 3.1.1 Test for immunoglobulin content

When the Cellulose Acetate Membrane Electrophoretic Test given in General Tests is applied, no less than 95% of the total proteins shall be immunoglobulin.

##### 3.1.2 Test for freedom from residual proteolytic enzyme

When measured by a suitable method for the determination of proteolytic enzyme, the test material shall be practically free from residual proteolytic enzyme.

##### 3.1.3 Sterility test

The sterility test given in General Tests shall apply.

##### 3.1.4 Pyrogen test

The test given in General Tests shall apply.

#### 3.1.5 Test for antitoxin content

The method given in 3.2.7 shall apply.

### 3.2 Tests on final product

Following tests shall apply to each final lot.

#### 3.2.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### 3.2.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

#### 3.2.3 Test for protein content

When the test for protein nitrogen content given in General Tests is applied, the total protein content of a polyvalent product shall be no higher than 160 mg/mL, and shall be no higher than 140 mg/500 units of type A, B and E antitoxins, and per 200 units of type F antitoxin. In the case of a monovalent product, the protein content shall be no higher than 30 mg/500 units of type A, B or E antitoxin, and no higher than 50 mg/200 units of type F antitoxin.

#### 3.2.4 Sterility test

The test given in General Tests shall apply.

#### 3.2.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.2.6 Pyrogen test

The test given in General Tests shall apply.

#### 3.2.7 Potency test

##### 3.2.7.1 Materials

Potency test shall be conducted on test samples using Standard Antitoxins (hereafter referred to as "Standard" in this monograph) and test toxins corresponding to each antitoxin. In the case of type B antitoxin, toxins from proteolytic and nonproteolytic strains of *Clostridium botulinum* are used as test toxins. Dilution of these materials shall be made with a 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

##### 3.2.7.2 Test procedures

Potency test shall be conducted with respect to each antitoxin separately.

Each Standard shall be diluted to five levels at appropriate intervals determined with careful consideration to the accuracy of the test, and contain 0.050 unit/0.25mL for the median dilution (hereafter referred to as "standard dilution" in this monograph).

Similarly, a series of five dilutions shall be made with the test sample (hereafter referred to as "test dilution" in this monograph).

Each test toxin shall be diluted to a concentration of one test dose per 0.25 mL (hereafter referred to as "toxin dilution" in this monograph).

A volume shall be taken accurately from each of the standard and test dilutions, combined with an equal volume of corresponding toxin dilution and mixed well. Each mixture shall be kept standing for 1 hour and then injected intraperitoneally at a dose of 0.5 mL into at least 4 mice aged 23–29 days. The animals shall be observed for 3 days after injection.

##### 3.2.7.3 Criterion for judgment

The content of each antitoxin of the test sample shall be determined individually by statistical analysis of test results. The final product shall contain each antitoxin at no less than the value stated

on the label.

#### 3.2.8 Identity test

The test shall be conducted by methods appropriate to each of the respective antitoxins.

#### **4. Storage and expiry date**

The expiry date shall be 10 years.

#### **5. Other requirements**

##### 5.1 Modification of the proper name

In the case of monovalent, bivalent, or trivalent product, the name of the type of antitoxin contained in the product shall be added to the proper name, e.g., Freeze-dried Botulism Antitoxin (type E), Freeze-dried Botulism Antitoxin (types A and B) or Freeze-dried Botulism Antitoxin (types A, B and F). The tetravalent product for types A, B, E and F shall be named Freeze-dried Botulism Antitoxin.

##### 5.2 Antitoxin contents of final containers

A sealed final container shall contain each antitoxin in a quantity of no less than 10,000 units with respect to types A, B and E, and no less than 4,000 units with respect to type F.

##### 5.3 Labeling

- (1) The name of the type of antitoxin contained in the product
- (2) Antitoxin content in units per mL with respect to each antitoxin after reconstitution.

# LIVE ORAL POLIOMYELITIS VACCINE

## 1. Descriptive definition

“Live Oral Poliomyelitis Vaccine” is a slightly yellow or pink clear liquid product containing live attenuated poliovirus of types 1, 2 and 3 (hereafter referred to as "virus" in this monograph). When frozen, the product may be slightly yellow to slightly pink with a whitish tint. When thawed, the product may contain educts of tissue protein or gelatin. Monovalent or bivalent product may be prepared, if necessary.

## 2. Production control

### 2.1 Source materials

#### 2.1.1 Strains of virus used for production

Strain LS-c, 2ab of type 1, strain P712, Ch, 2ab of type 2 and strain Leon, 12a<sub>b</sub> of type 3 virus or other strains approved to be adequate for the product shall be used. The virus contained in the product shall not have undergone more than five passages from the original vaccine approved as an adequate strain for production.

#### 2.1.2 Animals and kidneys used for production

Kidneys from *Cercopithecus* monkeys or other species with equal sensitivity shall be used. Animals shall be kept in quarantine groups with one group per room, and individually in cages closed on all sides except the front side. No animal from one cage shall be replaced with another animal from another cage. The quarantine period shall be at least 6 weeks. Animals shall be tuberculin-negative and negative for both anti-SV40 and anti-SIV antibodies. If any abnormal sign is observed in any animal during the quarantine period, none of the animals in that affected animal's quarantine group shall be used for vaccine production until the cause of the abnormal sign has been clarified and shown not to impair the safety of the vaccine produced. If the monthly death rate of animals in a quarantine group reaches 5% or higher, the animals shall be subjected to a new quarantine for 6 weeks, unless it becomes evident that the specifically determined case does not impair the safety of the product. When animals are killed for the removal of kidneys, the animal shall be thoroughly examined by autopsy. Only the kidneys of animals showing no pathological lesions, no matter whether related or irrelevant to the use of the kidneys for the production of vaccine, shall be used. The kidneys from animals used for other experiments shall not be used.

### 2.2 Bulk material

#### 2.2.1 Kidney-cell cultures

Kidney cells from individual animals shall be cultured separately. A minimal amount of antibiotics may be added to the cultures. However, penicillin and  $\beta$ -lactam antibiotics shall not be used. Prior to inoculation with virus, each cell culture shall be examined, and no cytopathic change shall be detected. The individual cell cultures shall be subjected to the tests given in 3.1.

#### 2.2.2 Virus suspensions

##### 2.2.2.1 Single harvest

The individual cell cultures shall be inoculated with virus, and virus suspensions from the individual cell cultures shall be harvested after a period of not longer than 4 days. The temperature

of incubation shall be in the range of 33°C to 35°C with the range of variation not greater than  $\pm 0.5^\circ\text{C}$ . Phenol red shall be added to the maintenance medium of cells at a concentration of not higher than 0.002 w/v%. A minimal amount of antibiotics, but no serum or fractions thereof nor penicillin shall be added. The single harvest shall be subjected to the tests given in 3.2.1.

#### 2.2.2.2 Monovalent bulk suspension before filtration

Single harvests of a strain shall be pooled to make a monovalent bulk suspension before filtration. Appropriate stabilizer may be added to the monovalent bulk suspension before filtration. The monovalent bulk suspension before filtration shall be subjected to the tests given in 3.2.2.

#### 2.2.3 Filtered monovalent bulk suspension

Monovalent bulk suspension shall be filtered to serve as the filtered monovalent bulk suspension. The filtered monovalent bulk suspension shall be subjected to the tests given in 3.3.

### 2.3 Final bulk

Filtered monovalent bulk suspensions shall be appropriately mixed and combined with sterile solutions of purified sucrose to make the final bulk with the indicated virus contents in 35w/v% sucrose solution. The final bulk shall be subjected to the tests given in 3.4.

## 3. Control tests

### 3.1 Control tests on cell cultures

#### 3.1.1 Tests on growth medium of individual cell cultures

The growth medium shall be removed and pooled to serve as the test sample. The test sample shall be subjected to the tests given in 3.1.3, 3.1.4 and 3.1.5. The ratio of volumes of the test sample to the maintenance medium of cell cultures shall be 1:1–1:3, and the area of the cell sheet shall be at least 3 cm<sup>2</sup>/mL of the test sample.

#### 3.1.2 Tests on control cell cultures

Either a portion of 25% or a maximum of 2500 mL of the individual cell cultures shall be left uninoculated to serve as the control cell cultures. The control cell cultures shall be incubated under the same conditions as the inoculated cultures for an observation period of 14 days, and examined for any cytopathic change due to extraneous virus. During the observation period, cultures discarded due to any nonspecific or incidental reason shall be less than 20% of the total cultures. On the seventh day and at the end of the observation period, the pooled sample of the maintenance medium from each culture shall be subjected to the tests given in 3.1.3, 3.1.4 and 3.1.5. The maintenance medium shall be removed from 4% of the control cell cultures on the fourth day and from all the remaining cultures at the end of the observation period. The test given in 3.1.6 shall be applied to those cultures deprived of the maintenance medium.

#### 3.1.3 Test in *Cercopithecus* monkey kidney-cell cultures

A 10-mL portion of the test sample shall be inoculated into kidney-cell cultures from *Cercopithecus* monkeys, which shall be incubated at  $36.0 \pm 0.5^\circ\text{C}$  for an observation period of 14 days. No cytopathic change due to SV40 or other extraneous virus shall be detected. At the end of the observation period, the culture fluid shall be further transferred to other cell cultures, which shall be incubated for another observation period of 14 days. No cytopathic change due to extraneous virus shall be detected. This second test may be omitted in the test of the control cell cultures.

#### 3.1.4 Test in rabbit kidney cell cultures

A 10-mL portion of the test sample shall be inoculated into rabbit kidney cell cultures, and incubated at  $36.0 \pm 0.5^{\circ}\text{C}$  for an observation period of 14 days. No cytopathic change due to B virus or other extraneous virus shall be detected.

#### 3.1.5 Test in human cell cultures

A 10-mL portion of the test sample shall be inoculated into human cell cultures, and incubated at  $36.0 \pm 0.5^{\circ}\text{C}$  for an observation period of 14 days. No cytopathic change due to measles virus or other extraneous virus shall be detected. The test may be conducted in other cell cultures with an equal susceptibility to measles virus.

#### 3.1.6 Test for freedom from hemadsorption viruses

When the test sample listed in 3.1.2 is tested by the addition of red blood cells of guinea pigs, no hemadsorption virus shall be detected.

### 3.2 Tests on virus suspensions

#### 3.2.1 Tests on single harvest

The requirements given in 3.1.3 shall be applied to 5mL, those given in 3.1.4 to 20 mL, and those in 3.1.5 to 5 mL of the test sample. The test sample to be subjected to the tests given in 3.1.3 and 3.1.5 shall be treated with type-specific anti-poliovirus immune serum to neutralize the virus. The immune serum used for the neutralization of virus in this test shall be prepared by immunizing animals other than monkeys with polioviruses propagated in non-simian cells.

#### 3.2.2 Tests on monovalent bulk suspension before filtration

##### 3.2.2.1 Sterility test

Sterility Test, Mycoplasma Test, Mycobacterial Sterility Test given in General Tests shall apply. For the Mycobacterial Sterility Test, a 25-mL portion of the test sample shall be centrifuged and resuspended in 5 mL of physiological saline prior to use.

##### 3.2.2.2 Tests for freedom from extraneous viruses

###### 3.2.2.2.1 Test in adult mice

The test sample shall be given by intraperitoneal injection at a dose of 0.5 mL and intracerebral injection at a dose of 0.03 mL into at least 20 mice aged 5 weeks. The inoculated animals shall be observed for 21 days. No animal shall show signs of infection with lymphocytic choriomeningitis virus or other extraneous viruses, and at least 80% of the animals shall survive the observation period.

###### 3.2.2.2.2 Test in suckling mice

The test sample shall be given by intraperitoneal injection at a dose of 0.1 mL and intracerebral injection at a dose of 0.01 mL into at least 20 mice within 24 hours after birth. The inoculated animals shall be observed for 14 days. No animal shall show signs of infection with coxsackieviruses or other viruses, and at least 80% of the animals shall survive the observation period.

###### 3.2.2.2.3 Intracerebral test in guinea pigs

The test sample shall be given by intracerebral injection at a dose of 0.1 mL into at least 5 guinea pigs weighing 300–400 g. The inoculated animals shall be observed for 14 days. No animal shall show signs of infection with extraneous viruses, and at least 80% of the animals shall survive the observation period.

#### 3.2.2.2.4 Intraperitoneal test in guinea pigs

The test sample shall be given by intraperitoneal injection at a dose of 5 mL into at least 5 guinea pigs weighing 300–400 g. The incubated animals shall be observed for 42 days. No animal shall show signs of infection with *Mycobacterium tuberculosis* and at least 80% of the animals shall survive the observation period.

#### 3.2.2.2.5 Test in rabbits

The test sample shall be injected into at least 10 rabbits weighing 1.5–2.5 kg intradermally at multiple sites at a dose of 1mL and subcutaneously at a dose of 9mL. The inoculated animals shall be observed for 21 days. No animal shall show signs of infection with B virus or other extraneous viruses, and at least 80% of the animals shall survive the observation period.

### 3.3 Control tests on filtered monovalent bulk suspensions

#### 3.3.1 Sterility test

The test given in General Tests shall apply.

#### 3.3.2 Test for identification of type of virus

The test shall be conducted to identify the type of virus contained in the test sample with immune serum specific to each type of virus.

#### 3.3.3 Test for virus content

The test sample shall be made to appropriate logarithmic serial dilutions. Each dilution shall be inoculated into adequate cell cultures, which shall be incubated to determine the virus content. The virus content shall be no lower than  $10^{7.5}$  CCID<sub>50</sub>/mL.

#### 3.3.4 Test for rct/40 marker

Each of the test samples and Reference Virus of the same type shall be serially diluted. Each dilution shall be inoculated into cell cultures, which shall be incubated to determine the ratio of re-productive capacity of virus at  $36.0 \pm 0.5^\circ\text{C}$  to that at  $40.0 \pm 0.1^\circ\text{C}$ . The ratio for virus of type 1 shall be no less than  $10^6$  and those for viruses of type 2 and type 3 shall be no less than  $10^5$ . When the test is carried out in tube cultures, culture inoculated with type 3 virus can be incubated at  $40.3 \pm 0.1^\circ\text{C}$  instead of  $40.0 \pm 0.1^\circ\text{C}$ . When the test is conducted on the Reference Virulent Virus or other virulent viruses with equivalent virulence, the ratio of re-productive capacity at the specified temperatures shall be not greater than 100.

#### 3.3.5 Test for d-marker

The test sample and the Reference Virus of the same type shall be serially diluted, and the tests for d-marker shall be conducted. The propagation of the virus on agar medium containing a low concentration of sodium hydrogen carbonate shall be slower than that on agar medium containing a high concentration of sodium hydrogen carbonate, and the ratio of propagation shall be not be less than 1:100. When the test is conducted on the Reference Virulent Virus or other virulent viruses with equivalent virulence, the ratio shall be not greater than 1:10.

#### 3.3.6 Neurovirulence test

##### 3.3.6.1 Materials

The test sample and the Reference Virus of the same type shall be used. Each shall be diluted to make a virus content of  $10^{7.0}$  CCID<sub>50</sub>/mL, and further diluted serially to 10-, 100-, 1,000- and 10,000-fold dilutions (hereafter referred to as "dilution" in this monograph).

### 3.3.6.2 Test procedures

*Macaca* monkeys weighing at least 1.5 kg and tuberculin-negative shall be used after quarantine for a period of at least 6 weeks. The interval between the inoculation of the groups of monkeys with the test samples and with the Reference Virus of the same type shall be not greater than 3 months. For the tests on poliovirus type 1 and type 2 vaccines, the number of monkeys inoculated for the homotypic reference or the test vaccine shall be no less than 11. For the test on type 3 poliovirus vaccine, the number of monkeys inoculated for the Reference or the test vaccine shall be no less than 18. An input dose of virus within the range  $10^{6.5}$ – $10^{5.5}$  CCID<sub>50</sub> in 0.1 mL shall be injected into the gray matter of the lumbar spinal cord. Inoculation with other dilutions may be added to the test when considered necessary. The monkeys shall be observed for a period of at least 18 days, and paralysis caused by mechanical damage brought by injection and other clinical signs such as paralysis shall be recorded. At the end of the observation period, at least 80% of the monkeys shall survive. In addition, animals who may indicate an immunodeficiency status, as suggested by clinical signs and symptoms and histopathological findings, or who may show immunologic diseases as an underlying disease, shall be excluded from the test. Histological examinations shall be made on the lumbar cord, cervical cord, lower and upper medulla, as well as midbrain of all monkeys. In the histological examinations, the monkeys with evidence of the injection at the gray matter of lumbar cord shall be observed for pathological changes.

### 3.3.6.3 Criterion for judgment

No apparent difference shall be detected between the test sample and Reference Virus of the same type in overall results on the character, severity, and extent of histological lesions and clinical signs.

## 3.4 Tests on final bulk

### 3.4.1 Sterility test

The test given in General Tests shall apply.

## 3.5 Control tests on final product

Following tests shall apply to each final lot.

### 3.5.1 Sterility test

The test given in General Tests shall apply.

### 3.5.2 Test for virus content

The test sample shall be treated with immune sera against each type of poliovirus other than the type to be tested to determine the virus content of the particular type. The virus content of each type shall be in accordance with that stated on the label.

### 3.5.3 Test for thermal stability

The final product shall be treated for 48 hours at 37°C. The total virus content shall be compared with that of the final product kept at -20°C. The decrease in total virus content due to heating shall be no less than  $10^{0.5}$ .

### 3.5.4 Confirmation of the labeling

The test sample shall be treated with immune sera against each type of poliovirus other than the type to be tested to determine the virus content of the particular type. The virus content of each type shall be in accordance with that stated on the label.

#### **4. Storage and dating period**

Storage temperature shall be -20°C or less. The expiry date shall be two years.

#### **5. Other requirements**

##### 5.1 Modification of the proper name

For monovalent or divalent products, the type of viruses contained shall be added to the proper name.

##### 5.2 Labeling

(1) Virus content of each type

(2) Caution that the product is for oral use only

##### 5.3 Information to be provided in package insert and other labeling

(1) Name of the strain used for production

(2) Name and amount of antibiotics used in the cultivation of virus, if applicable

(3) Recommended human dose and route of administration, as follows:

The product shall be administered orally in 2 doses of 0.05 mL at an interval of 6 weeks or longer.

# **FREEZE-DRIED LIVE ATTENUATED MEASLES VACCINE**

## **1. Descriptive definition**

“Live Attenuated Measles Vaccine” is a freeze-dried product containing attenuated measles virus (hereafter referred to as "virus" in this monograph). When reconstituted, it becomes a colorless or reddish clear liquid.

## **2. Production control**

### 2.1 Source materials

#### 2.1.1 Strains of virus used for production

Strains of virus approved as suitable for production shall be used. Virus contained in the product shall be passaged under the approved culture conditions and shall not be passaged more than 5 times from the original vaccine approved as adequate for the strain for production.

#### 2.1.2 Embryonated hen eggs used for production

Chick embryos used for virus cultivation shall be harvested from chicken eggs derived from healthy flocks free from infectious diseases.

#### 2.1.3 Culture media

The cell culture medium can be supplemented with appropriate cell growth-promoting substances, phenol red at a concentration not higher than 0.002 w/v%, and minimal amounts of antibiotics, except penicillin. If non-human serum or fractions thereof are added to the medium, serum albumin concentration in the final bulk shall be controlled to be no higher than 50 ng per dose. The culture medium used for the propagation of virus can be supplemented with phenol red at a concentration not higher than 0.002 w/v%, appropriate stabilizer and minimal amounts of antibiotics. However, no non-human serum or fractions thereof, nor penicillin shall be added.

### 2.2 Bulk material

#### 2.2.1 Cell culture

Chick embryonic cell culture obtained in one session shall be regarded as an “individual cell culture”. No cytopathic change shall be detected prior to the inoculation of virus. The individual cell culture shall be subjected to the test given in 3.1.

#### 2.2.2 Virus suspension

Chick embryonic cell culture shall be used for propagation. The virus suspension harvested from individual cell cultures shall be pooled to serve as a single harvest. The single harvest shall be tested as directed in 3.2.1. The single harvests shall be pooled to make the virus suspension. Appropriate stabilizer may be added to the virus suspension before filtration. The virus suspension before filtration shall be tested as directed in 3.2.2

#### 2.2.3 Filtration

The virus suspension before filtration shall be centrifuged and/or filtrated to eliminate cells, and the suspension shall be combined to make the bulk material. The bulk material shall be tested as directed in 3.3.

### 2.3 Final bulk and freeze-drying

The bulk material shall be diluted, if required, to make the final bulk. Appropriate stabilizer may be used. However, antibiotics shall not be added. The final bulk shall be filled into final containers and freeze-dried. The final bulk shall be tested as directed in 3.4.

### **3. Control tests**

#### **3.1 Tests on cell cultures derived from chick embryos**

A volume equivalent to 25% or 500 mL of individual cell cultures shall be used as the control cell culture of uninfected cells. The control cell culture shall be tested as given below.

##### **3.1.1 Observation of cell culture**

The control cell cultures shall be cultivated without being inoculated with the virus under the same conditions used for virus infected cell cultures. No cytopathic change due to extraneous virus(s) shall be detected. During the observation period, control cell cultures discarded due to any nonspecific or incidental reason shall be less than 20% of the total control cells cultures.

##### **3.1.2 Tests in cell cultures**

At the end of the above observation period, the culture medium shall be harvested from the vessel, pooled if necessary, and be tested as directed in 3.3.3.2

#### **3.2 Tests on virus suspension**

##### **3.2.1 Tests on single harvest**

###### **3.2.1.1 Sterility test**

Sterility Test and Mycoplasma Test given in General Tests shall apply.

###### **3.2.1.2 Tests for freedom from extraneous viruses**

The tests given in 3.3.3.2 shall apply. In this case, the tests shall be performed, if necessary, after the neutralization of virus with anti-measles serum. The antiserum for the neutralization of virus shall be prepared in any suitable animal except humans, monkeys and chickens.

##### **3.2.2 Tests on virus suspension before filtration**

###### **3.2.2.1 Sterility test**

Sterility Test, Mycoplasma Test, Mycobacterial Sterility Test given in General Tests shall apply. For the Mycobacterial Sterility Test, a 25-mL portion of the test sample shall be centrifuged and resuspended in 5 mL of physiological saline prior to use.

#### **3.3 Tests on bulk material**

The bulk material diluted to the same concentration as the final bulk shall be tested according to the procedures given below.

##### **3.3.1 Staining test**

The test given in General Tests shall apply.

##### **3.3.2 Sterility test**

The test given in General Tests shall apply.

##### **3.3.3 Test for freedom from extraneous viruses**

The test shall be performed after neutralization of measles virus, if necessary, according to the procedures given in 3.2.1.2.

### 3.3.3.1 Tests in animals

#### 3.3.3.1.1 Inoculation of adult mice

At least 10 mice aged 4–5 weeks shall be inoculated intraperitoneally with 0.5 mL and intracerebrally with 0.03 mL of the sample. The mice shall be observed for 21 days. No animal shall show evidence of infection with extraneous microbial agents, and more than 80% of the inoculated mice must survive the observation period.

#### 3.3.3.1.2 Inoculation of suckling mice

Within 24 hours after birth, suckling mice shall be inoculated intraperitoneally with 0.1 mL and intracerebrally with 0.01 mL of the test sample. The mice shall be observed for 14 days. Except those died within the first 24 hours of the test, no mouse of at least 20 mice shall show evidence of infection with extraneous microbial agents, and more than 80% of the mice must survive the observation period.

#### 3.3.3.1.3 Intracerebral inoculation of guinea pigs

At least 5 guinea pigs of 300–400 g weight shall be inoculated intracerebrally with 0.1 mL of the test sample. The guinea pigs shall be observed for 14 days. No guinea pig shall show evidence of infection with extraneous microbial agents, and more than 80% of the guinea pigs must survive the observation period.

### 3.3.3.2 Tests in cell cultures

#### 3.3.3.2.1 Inoculation of human tissue culture cells

A 10-mL portion of the test sample shall be inoculated into human cells. The inoculated cells shall be cultured for 7 days, and portions of the cells shall be passaged by culture for a further 7 days. No cytopathic change due to extraneous viruses shall be detected within the entire culture period.

#### 3.3.3.2.2 Inoculation of primary tissue cultures of embryonic chick cells

A 25-mL portion of the test sample shall be inoculated into primary cultures of chick embryo cells. The inoculated cells shall be cultured and passaged 3 times. No avian leukemia viruses shall be detected in the cultured cells or fluid using an approved method such as enzyme-linked immunosolvent assay. In addition, no reticuloendotheliosis virus antigen shall be stained immunologically in the cultured cells using the anti-reticuloendotheliosis virus serum.

#### 3.3.3.2.3 Inoculation of primary renal tissue cultures of chick cells

A 5-mL portion of the test sample shall be inoculated into primary cultures of chick kidney cells. Inoculated cells shall be incubated and observed for 14 days. Then cells shall be frozen and thawed to induce passage, and subsequently inoculated into fresh primary cultures of chick kidney cells. After observation for 14 days, the inoculated cells shall be examined by the cell-hemadsorption assay with red blood cells of guinea pigs and chickens. No cell-hemadsorption shall be detected. No cytopathic change due to extraneous viruses shall be detected during any observation period.

#### 3.3.3.3 Inoculation of embryonated chicken eggs

At least 20 embryonated chicken eggs aged 10–11 days each inoculated with 0.25 mL of test sample onto the chorioallantoic membranes or into the allantoic cavity shall be incubated for 3 days. At least 20 embryonated chicken eggs aged 6–7 days each inoculated with 0.25 mL of the test sample into the yolk sac shall be incubated for 12 days. No egg, except those died within the first 24 hours of the test, shall show evidence of infection with extraneous viruses, and more than 80% of the eggs must survive the observation period. Samples obtained from eggs that died after the first 24

hours of the test shall be reinoculated into at least 10 additional eggs by the same inoculation route, and these should be incubated for the same number of days. No egg shall show evidence of infection with extraneous viruses, and more than 80% of the eggs must survive the observation period.

#### 3.3.4 Identification test

The propagation of virus in the sample shall be suppressed in appropriate cell cultures after neutralization with anti-measles virus serum.

#### 3.3.5 Test for confirmation of attenuation of virus

The test shall be conducted using *Macacus* or *Cercopithecus* monkeys that have been proven to be serologically negative for measles. At least 15 monkeys shall be employed in this test. The test sample shall be given by injection of 0.5 mL into the thalamic region of each hemisphere, 0.25 mL into cerebellomedullary cistern and 1.0 mL subcutaneously. Seven days after injection, one-third of the monkeys shall be killed by autopsy. There shall be no typical lesions induced by wild measles virus in the monkey tissues. The rest of the monkeys shall be observed for 21 days after injection.

During the observation period, none of the monkeys shall show symptoms of paralysis or other evidence of neurological involvement, and more than 80% of the monkeys must survive. There shall be no cases that show abnormal symptoms or death due to the inoculated virus or extraneous virus in the inoculum. Upon autopsy at the end of the observation period, there shall be no abnormal lesions in the central nervous system due to the inoculated virus or extraneous virus in the inoculum. However, monkeys that show clinical or pathological evidence suggesting immunodeficiency or which have underlying immune disorders shall be excluded from the study. Furthermore, no less than 80% of the monkeys shall be serologically positive for measles. Four uninoculated monkeys shall be maintained as a control, 2 monkeys as cage mates, the other 2 within the same area as the inoculated monkeys. Those control monkeys shall be observed for 21 days. The control monkeys must show no clinical signs or the development of anti-measles virus antibody in the serum at the end of the observation period. There shall be no clear difference in test results (i.e., character, degree and range of the pathological changes, symptoms during the observation period) between consecutive lots of bulk material prepared from a seed virus strain.

If each of the first 5 consecutive lots of the bulk material prepared from a seed virus strain satisfies the requirements given above, subsequent lots prepared from the same seed virus strain are not required to undergo the test for confirmation of the attenuation of virus.

#### 3.3.6 Test for virus content

The test given in 3.5.3 shall apply.

### 3.4 Tests on final bulk

#### 3.4.1 Staining test

The test given in General Tests shall apply.

#### 3.4.2 Sterility test

The test given in General Tests shall apply.

#### 3.4.3 Test for virus content

The final bulk shall be tested for measles virus concentration according to the procedure given in 3.5.3

#### 3.4.4 Test for freedom from abnormal toxicity

The final bulk shall be tested for the absence of abnormal toxicity according to the procedure given in General Tests.

### 3.5 Tests on final product

Following tests shall apply to each final lot.

#### 3.5.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the procedures given in General Tests.

#### 3.5.2 Sterility test

The final product shall be tested for bacterial and mycotic sterility according to the procedure given in General Tests.

#### 3.5.3 Potency test

The concentration of mumps virus in the final product shall be determined by titration in a suitable cell culture system. The potency of the test sample shall be shown as PFU, FFU, or CCID<sub>50</sub>. The minimum acceptable virus titer per 0.5 mL shall be 5,000.

#### 3.5.4 Identity test

Measles virus in the final product shall be identified by immunofluorescence after propagation of virus in an appropriate cell culture.

## 4. Storage and expiry date

The final product shall be kept at 5 °C or less during storage. The expiry date shall be one year.

## 5. Other requirements

### 5.1 Reconstituent

The reconstituent shall be water for injection.

### 5.2 Information to be provided in package insert and other labeling

- (1) Name of the virus strain used for production.
- (2) Name of the cell culture used for the cultivation of virus.
- (3) Names and concentrations of antibiotics or dyes used in the cultivation of virus, if applicable.
- (4) Names and concentrations of stabilizer, if used.
- (5) The recommended human dosage and route of administration is as follows:

Usually, 0.5 mL is given by subcutaneous injection.

# **FREEZE-DRIED LIVE ATTENUATED MEASLES, MUMPS, RUBELLA COMBINED VACCINE**

## **1. Descriptive definition**

“Freeze-dried Live Attenuated Measles, Mumps, Rubella Combined Vaccine” is a freeze-dried product of a trivalent mixture containing a suitable strain of live attenuated measles virus (hereafter referred to as “measles virus” in this monograph), live attenuated mumps virus (hereafter referred to as “mumps virus” in this monograph), and live attenuated rubella virus (hereafter referred to as “rubella virus” in this monograph). When reconstituted, it becomes a colorless or reddish clear liquid.

## **2. Production control**

### **2.1 Source materials**

For source materials of individual components, the procedures provided in Freeze-dried Live Attenuated Measles Vaccine 2.1 (for measles virus), Freeze-dried Live Attenuated Mumps Vaccine 2.1 (for mumps virus), and Freeze-dried Live Attenuated Rubella Vaccine 2.1 (for rubella virus) shall be applied, respectively.

### **2.2 Bulk material**

For the bulk material of individual components, the procedures provided in Freeze-dried Live Attenuated Measles Vaccine 2.2 (for measles virus), Freeze-dried Live Attenuated Mumps Vaccine 2.2 (for mumps virus), and Freeze-dried Live Attenuated Rubella Vaccine 2.2 (for rubella virus) shall be applied, respectively.

### **2.3 Final bulk and freeze-drying**

Individual bulk material of Freeze-dried Live Attenuated Measles Vaccine, Freeze-dried Live Attenuated Mumps Vaccine, and Freeze-dried Live Attenuated Rubella Vaccine shall be blended with an appropriate quantity, and diluted if necessary, to make the final bulk. Appropriate stabilizer may be used. However, antibiotics shall not be added. The final bulk shall be dispensed into final containers and freeze-dried. The final bulk shall be subjected to the test given in 3.4.

## **3. Control tests**

### **3.1 Tests on individual cell cultures**

For tests on cell cultures of individual components, the procedures provided in Freeze-dried Live Attenuated Measles Vaccine 3.1 (for measles virus), Freeze-dried Live Attenuated Mumps Vaccine 3.1 (for mumps virus), and Freeze-dried Live Attenuated Rubella Vaccine 3.1 (for rubella virus) shall be applied, respectively.

### **3.2 Tests on virus suspension**

For control tests on virus suspensions of individual components, the procedures prescribed in Freeze-dried Live Attenuated Measles Vaccine 3.2 (for measles virus), Freeze-dried Live Attenuated Mumps Vaccine 3.2 (for mumps virus), and Freeze-dried Live Attenuated Rubella Vaccine 3.2 (for rubella virus) shall be applied, respectively.

### **3.3 Tests on bulk material**

For control tests on bulk material of individual components the procedures prescribed in Freeze-dried Live Attenuated Measles Vaccine 3.3 (for measles virus), Freeze-dried Live Attenuated Mumps Vaccine 3.3 (for mumps virus), and Freeze-dried Live Attenuated Rubella Vaccine 3.3 (for rubella virus) shall be applied respectively.

#### 3.4 Tests on final bulk

##### 3.4.1 Staining test

The test given in General Tests shall apply.

##### 3.4.2 Sterility test

The test given in General Tests shall apply.

##### 3.4.3 Test for virus content

The test given in 3.5.3 shall apply.

##### 3.4.4 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.5 Tests on final product

Following tests shall apply to each final lot.

##### 3.5.1 Test for moisture content

Moisture content shall not be higher than 3.0% when tested according to the test given in General Tests.

##### 3.5.2 Sterility test

The test given in General Tests shall apply.

##### 3.5.3 Potency test

The concentration of individual measles, mumps and rubella viruses in the final product after reconstitution with the accompanying solvent shall be determined by titration in suitable cell culture systems. The potency of the test sample shall be shown as PFU, FFU, or CCID<sub>50</sub>. The minimum acceptable virus titer per 0.5 ml shall not be less than 5,000 for measles and mumps viruses, and 1,000 for rubella virus, respectively.

##### 3.5.4 Identity test

Measles, mumps, and rubella viruses in the final product shall be identified individually by immunofluorescence methods after propagation of virus in appropriate cell cultures.

#### **4. Storage and expiry date**

The final product shall be kept at 5 °C or less during the storage. The expiry date shall be one year.

#### **5. Other requirements**

##### 5.1 Reconstituent

The reconstituent shall be water for injection.

##### 5.2 Information to be provided in package insert and other labeling

(1) Name of the virus strain used for production

- (2) Name of the cell culture used for the cultivation of virus
- (3) Names and amounts of antibiotics or dyes used in the cultivation of virus, if applicable
- (4) Names and amounts of stabilizer, if used
- (5) Recommended human dosage and route of administration, as follows:  
Usually, 0.5 ml is given by subcutaneous injection.

# **FREEZE-DRIED LIVE ATTENUATED MEASLES, RUBELLA COMBINED VACCINE**

## **1. Descriptive definition**

“Freeze-dried Live Attenuated Measles, Rubella Combined Vaccine” is a freeze-dried product of a bivalent mixture containing a suitable strain of live attenuated measles virus (hereafter referred to as “measles virus” in this monograph), and live attenuated rubella virus (hereafter referred to as “rubella virus” in this monograph). When reconstituted, it becomes a colorless or reddish clear liquid.

## **2. Production control**

### 2.1 Source materials

For source materials of individual components, the procedures provided in Freeze-dried Live Attenuated Measles Vaccine 2.1 (for measles virus), and Freeze-dried Live Attenuated Rubella Vaccine 2.1 (for rubella virus) shall be applied, respectively.

### 2.2 Bulk material

For the bulk material of individual components, the procedures provided in Freeze-dried Live Attenuated Measles Vaccine 2.2 (for measles virus), and Freeze-dried Live Attenuated Rubella Vaccine 2.2 (for rubella virus) shall be applied, respectively.

### 2.3 Final bulk and freeze-drying

Individual bulk material of Freeze-dried Live Attenuated Measles Vaccine, and Freeze-dried Live Attenuated Rubella Vaccine shall be blended with an appropriate quantity, and diluted if necessary, to make the final bulk. Appropriate stabilizer may be used. However, antibiotics shall not be added. The final bulk shall be filled into final containers and freeze-dried. The final bulk shall be subjected to the test given in 3.4.

## **3. Control tests**

### 3.1 Tests on individual cell cultures

For tests on cell cultures of individual components, the procedures provided in Freeze-dried Live Attenuated Measles Vaccine 3.1 (for measles virus), and Freeze-dried Live Attenuated Rubella Vaccine 3.1 (for rubella virus) shall be applied, respectively.

### 3.2 Tests on virus suspension

For control tests on virus suspensions of individual components, the procedures prescribed in Freeze-dried Live Attenuated Measles Vaccine 3.2 (for measles virus), and Freeze-dried Live Attenuated Rubella Vaccine 3.2 (for rubella virus) shall be applied, respectively.

### 3.3 Tests on bulk material

For control tests on bulk material of individual components the procedures prescribed in Freeze-dried Live Attenuated Measles Vaccine 3.3 (for measles virus), and Freeze-dried Live Attenuated Rubella Vaccine 3.3 (for rubella virus) shall be applied respectively.

### 3.4 Tests on final bulk

#### 3.4.1 Staining test

The test given in General Tests shall apply.

#### 3.4.2 Sterility test

The test given in General Tests shall apply.

#### 3.4.3 Test for virus content

The test given in 3.5.3 shall apply.

#### 3.4.4 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.5 Tests on final product

Following tests shall apply to each final lot.

##### 3.5.1 Test for moisture content

Moisture content shall not be higher than 3.0% when tested according to the test given in General Tests.

##### 3.5.2 Sterility test

The test given in General Tests shall apply.

##### 3.5.3 Potency test

The concentration of individual measles, mumps and rubella viruses in the final product after reconstitution with the accompanying solvent shall be determined by titration in suitable cell culture systems. The potency of the test sample shall be shown as PFU, FFU, or CCID<sub>50</sub>. The minimum acceptable virus titer per 0.5 ml shall not be less than 5,000 for measles virus and 1,000 for rubella virus, respectively.

##### 3.5.4 Identity test

Measles, and rubella viruses in the final product shall be identified individually by immunofluorescence methods after propagation of virus in appropriate cell cultures.

#### **4. Storage and expiry date**

The final product shall be kept at 5°C or less during the storage. The expiry date shall be one year.

#### **5. Other requirements**

##### 5.1 Reconstituent

The reconstituent shall be water for injection.

##### 5.2 Information to be provided in package insert and other labeling

- (1) Name of the virus strain used for production
- (2) Name of the cell culture used for the cultivation of virus
- (3) Names and amounts of antibiotics or dyes used in the cultivation of virus, if applicable
- (4) Names and amounts of stabilizer, if used
- (5) Recommended human dosage and route of administration, as follows:

Usually, 0.5 ml is given by subcutaneous injection.

## **FREEZE-DRIED MAMUSHI ANTIVENOM, EQUINE**

### **1. Descriptive definition**

“Freeze-dried Mamushi Antivenom” is a freeze-dried product containing "Mamushi (*Agkistrodon halys*) antivenom" in immunoglobulin of horses (hereafter referred to as "antivenom" in this monograph). When reconstituted, it becomes a colorless or slightly yellowish brown, clear or slightly whitish turbid liquid.

### **2. Production control**

#### 2.1 Source materials

##### 2.1.1 Antigens used for immunization

The venom of Mamushi (*Agkistrodon halys*) or a toxoid derived from it shall be used for immunization.

##### 2.1.2 Animals used for production

Horses shall be used for production.

#### 2.2 Bulk material

##### 2.2.1 Crude antivenom material

Crude serum or plasma shall be used if it contains no less than 100 units of antivenom per mL with respect to anti-lethal toxin and anti-hemorrhagic toxin and passes the sterility test and the pyrogen test given in General Tests.

##### 2.2.2 Purification

The fraction containing immunoglobulin shall be prepared by fractionating the source plasma using a suitable method that has been shown not to cause the deterioration of antibodies. The fraction shall be treated with a proteolytic enzyme. The preparation containing the treated antitoxin shall serve as the bulk material.

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

#### 2.3 Final bulk and freeze-drying

The bulk material shall be diluted, if necessary, with buffered physiological saline or other suitable medium to contain no less than 300 units of antivenom per mL with respect to each anti-lethal and anti-hemorrhagic toxin, dispensed and freeze-dried.

### **3. Control tests**

#### 3.1 Tests on bulk material

##### 3.1.1 Test for immunoglobulin content

When the Cellulose Acetate Membrane Electrophoretic Test in General Tests is applied, no less than 95% of the total proteins shall be immunoglobulin.

##### 3.1.2 Test for freedom from residual proteolytic enzyme

When measured by a suitable method for the detection of proteolytic enzyme activity, the test material shall be practically free from residual proteolytic enzyme activity.

### 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.

### 3.1.5 Test for antivenom content

The test given in 3.2.7 shall apply.

## 3.2 Tests on final product

Following tests shall apply to each final lot..

### 3.2.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

### 3.2.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.8 to 7.4.

### 3.2.3 Test for protein content

When the test for protein nitrogen content given in General Tests is applied, the protein content shall be less than 30 mg per 300 units of the titer of either the antilethal toxin or anti-hemorrhagic toxin, whichever is lower.

### 3.2.4 Sterility test

The test given in General Tests shall apply.

### 3.2.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.2.6 Pyrogen test

The test given in General Tests shall apply.

### 3.2.7 Potency test

Potency shall be determined with respect to anti-lethal toxin and anti-hemorrhagic toxin.

#### 3.2.7.1 Determination of anti-lethal toxin titer

##### 3.2.7.1.1 Materials

The test sample, Standard Mamushi Antivenom (hereafter referred to as "Standard" in this monograph) and Mamushi Test Venom (lethal) shall be used. Dilution of these materials shall be made in 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

##### 3.2.7.1.2 Test procedures

The Standard shall be diluted to five levels at appropriate intervals determined with careful consideration to the accuracy of the test, containing 10.0 units per 0.1 mL for the median dilution (hereafter referred to as "standard dilution" in this monograph). A series of five dilutions shall be similarly made with the test sample (hereafter referred to as "test dilution" in this monograph).

Mamushi Test Venom (lethal) shall be diluted to contain one test dose per 0.1 mL (hereafter referred to as "venom dilution" in this monograph).

A volume shall be taken accurately from each of the Standard and test dilutions, combined with

an equal volume of venom dilution and mixed well. Each mixture shall be kept standing for 1 hour and injected intravenously at a dose of 0.2 mL into at least 4 mice aged 23–29 days. The animals shall be observed for 2 days after injection.

#### 3.2.7.1.3 Criterion for judgment

The titer for anti-lethal toxin of the test sample shall be determined by statistical analysis of test results.

The final product shall contain antivenom of no less than the value stated on the label.

#### 3.2.7.2 Determination of anti-hemorrhagic titer

##### 3.2.7.2.1 Materials

The test sample, Standard and Mamushi Test Venom (hemorrhagic) shall be used. Dilution of these materials shall be made with 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

##### 3.2.7.2.2 Test procedures

The Standard shall be diluted to five levels at appropriate intervals determined with careful consideration to the accuracy of the test, containing 1.0 unit/0.1 mL for the median dilution (hereafter referred to as "standard dilution" in this monograph). A series of five dilutions shall be similarly made with the test sample (hereafter referred to as "test dilution" in this monograph).

The test venom shall be diluted to contain one test dose per 0.1 mL (hereafter referred to as "venom dilution" in this monograph).

A volume shall be taken accurately from each of the Standard and test dilutions, combined with an equal volume of venom dilution and mixed well. Each mixture shall be kept standing for 1 hour and injected intracutaneously at a dose of 0.2 mL into rabbits weighing 2.0–3.0 kg. At least 2 injections at different sites shall be made with each mixture. Animals shall be killed by excess anesthesia 24 hours after injection and the skin shall be stripped off. The cross-diameter of hemorrhagic spots shall be measured from the inner side of the skin.

##### 3.2.7.2.3 Criterion for judgment

Anti-hemorrhagic titer of the test sample shall be determined by statistical analysis of the sizes of the hemorrhagic spots.

The final product shall contain antivenom of no less than the value stated on the label.

#### 3.2.8 Identity test

The test shall be conducted by suitable method.

### **4. Storage and expiry date**

The expiry date shall be 10 years.

### **5. Other requirements**

#### 5.1 Antivenom contents of final containers

A sealed final container shall contain antivenom of no less than 6,000 units with respect to each of the anti-lethal and anti-hemorrhagic toxin.

#### 5.2 Description on the label

Antivenom content in units per mL with respect to each antivenom

## WEIL'S DISEASE AND AKIYAMI COMBINED VACCINE

### 1. Descriptive definition

“Weil's Disease and Akiyami Combined Vaccine” is a liquid product containing inactivated Weil's disease leptospira, Akiyami A leptospira, Akiyami B leptospira, and Akiyami C leptospira (hereafter referred to as each "*leptospira*" in this monograph). It is possible that a product does not contain one or more *Akiyami leptospirae*.

### 2. Production control

#### 2.1 Source materials

##### 2.1.1 Strains of leptospira for production

Any adequate strain of leptospira of each serovar known to possess sufficient antigenicity shall be used. Strains for production shall be maintained by serial cultivations in Korthof's medium or other suitable medium with comparable efficiency.

##### 2.1.2 Culture media

Korthof's medium or other suitable medium shall be used.

#### 2.2 Bulk material

##### 2.2.1 Suspension of leptospira

After cultivation, the culture of leptospira free from any contaminant as shown by microscopic and other suitable culture examinations shall serve as a suspension of leptospira.

##### 2.2.2 Washing and inactivation of leptospira

Suspended leptospira shall be harvested by centrifugation, washed thoroughly with physiological saline or other suitable solution and resuspended. Inactivation shall be conducted by the addition of formaldehyde or other appropriate methods. The suspension containing washed and inactivated leptospira shall serve as the bulk material. The bulk material shall be subjected to the tests given in 3.1.

#### 2.3 Final bulk

The bulk material shall be diluted in buffered physiological saline or other suitable solution and mixed to render the approximate content of each leptospira as specified below when examined by the test given in 3.1.3.

|                           |                          |
|---------------------------|--------------------------|
| Weil's disease leptospira | $5 \times 10^8$ per mL   |
| Akiyami A leptospira      | $2.5 \times 10^8$ per mL |
| Akiyami B leptospira      | $2.5 \times 10^8$ per mL |
| Akiyami C leptospira      | $2.5 \times 10^8$ per mL |

Appropriate preservatives may be added.

### 3. Control tests

#### 3.1 Tests on bulk material

##### 3.1.1 Staining test

The test given in General Tests shall apply.

##### 3.1.2 Sterility test

The test given in General Tests shall apply.

#### 3.1.3 Test for leptospira content

Within 24 hours after resuspension and before inactivation, the dark-field microscopy and determination of optical density given in General Tests shall apply. In optical density determination, the density of suspensions of leptospira containing known numbers of leptospira per mL counted by the dark field microscopic method shall be used as a control to draw a calibration curve.

#### 3.1.4 Inactivation test

The test sample shall be inoculated at a dose of 0.5 mL into at least 3 tubes each containing 4.5 mL of Korthof's medium. The inoculated tube shall be incubated at  $29 \pm 1^\circ\text{C}$  for 14 days. After completion of the incubation period, each culture shall be examined by dark field microscopy. No live leptospira shall be detected in the test sample.

The test given in 3.2.7 shall also be applied to a sample diluted in 0.013 mol/L phosphate-buffered sodium chloride solution (pH 7.0) to give a leptospira concentration of  $2 \times 10^9/\text{mL}$ .

### 3.2 Tests on final product

#### 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 7.4.

#### 3.2.2 Test for protein nitrogen content

When the test given in General Tests is applied, the protein nitrogen content shall be no higher than 100  $\mu\text{g}/\text{mL}$ .

#### 3.2.3 Test for preservative content

When thimerosal is used as a preservative, the test given in General Tests shall apply. When phenol is used as a preservative, the test given in General Tests shall apply.

#### 3.2.4 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%.

#### 3.2.5 Sterility test

The test given in General Tests shall apply.

#### 3.2.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.2.7 Inactivation test

The test sample shall be inoculated at a dose of 0.5 mL into at least 3 tubes each containing 4.5 mL of Korthof's medium. The inoculated tubes shall be incubated at  $29 \pm 1^\circ\text{C}$  for 14 days. After completion of the incubation period, each culture shall be examined by dark field microscopy. No live leptospira shall be detected in the test sample. Also, the test sample shall be given by intraperitoneal injection at a dose of 5 mL into at least 2 guinea pigs weighing 300–400 g. The animals shall be observed for 14 days. No animal shall show fever, jaundice, conjunctival hyperemia, pronounced decrease in body weight or other abnormal signs.

#### 3.2.8 Identification of leptospira

The test shall be conducted in mice or guinea pigs.

##### 3.2.8.1 Identification test in mouse

The test sample shall be given by intraperitoneal injection into at least 10 mice aged 8 weeks at a dose of 0.1 mL. At 4–5 weeks after injection, serum of each animal shall be tested with each

serovar of leptospira intended for inclusion in the product. Each serum shall show positive results (>8 fold) for all serovars.

#### 3.2.8.2 Identification test in guinea pigs

The test sample shall be given by subcutaneous injection into at least 4 guinea pigs weighing 300–400 g in 2 doses of 1 mL at an interval of 1 week. At 14 days after the second injection, serum of each animal shall be tested with each serovar of leptospira intended for inclusion in the product. Each serum shall show positive results with all serovars.

#### 3.2.9 Potency test

Potency shall be measured by the serum agglutination test in mice or by intraperitoneal challenge in guinea pigs.

##### 3.2.9.1 Agglutination test

###### 3.2.9.1.1 Materials

The test sample, reference sample of the Weil's disease and Akiyami combined vaccine and the reference strains of leptospire for the agglutination test shall be used.

The test sample and reference sample shall be diluted with 0.013 mol/L phosphate-buffered sodium chloride solution (pH 7.0).

###### 3.2.9.1.2 Procedures

The test sample and the reference sample shall be serially diluted in a logarithmic scale. Each dilution of the test sample and reference sample must be injected at a dose of 1 mL into at least 10 mice aged 8 weeks. At 4 to 5 weeks after injection, serum of each animal shall be tested by the microscopic agglutination method with a reference strain of **Weil's disease leptospirae**.

###### 3.2.9.1.3 Criterion for judgment

The titer of the test sample shall be more than 3 units upon statistical analysis of test results.

##### 3.2.9.2 Intraperitoneal challenge

###### 3.2.9.2.1 Materials

The test sample and the **Weil's disease leptospirae** for challenge (hereafter referred to as "challenge strain" in this monograph) shall be used.

The challenge strain shall be inoculated into guinea pigs weighing 300–400 g. The liver of the animal at a moribund state showing typical jaundice at 6–8 days after injection shall be triturated in 0.013 M phosphate-buffered sodium chloride solution (pH 7.0) to make a 10 w/v% emulsion. The emulsion shall be centrifuged at  $1,900 \times g$  for 30 minutes and the supernatant shall be collected. The supernatant shall be diluted 10-fold to serve as the challenge suspension.

###### 3.2.9.2.2 Procedures

The test sample shall be given by subcutaneous injection into at least 8 guinea pigs weighing 300–400 g in 2 doses of 1 mL at an interval of 1 week. Each animal shall be given by intraperitoneal injection at 14 days after the second injection with 1mL of the challenge suspension. The animals shall be observed for 14 days after challenge.

As controls, the challenge suspension shall be given by intraperitoneal injection at a dose of 1mL into at least 8 non-immunized guinea pigs weighing 400–600 g. The animals shall be similarly observed. The animals shall die in 6–8 days, showing typical symptoms such as jaundice.

###### 3.2.9.2.3 Criterion for judgment

No less than 80% of the immunized animals shall survive.

### 3.2.10 Identity test

The test shall be conducted by agglutination or precipitation with immune serum against either of the respective serovars intended for inclusion.

## 4. Storage and expiry date

The expiry date shall be one year.

## 5. Other requirements

### 5.1 Modification of the proper name

Product lacking some or all of the *Akiyami* (leptospirae) shall be named accordingly, as in the following examples: Weil's Disease Vaccine (when no *Akiyami* (leptospirae) are contained), Weil's Disease and Akiyami A, B Vaccine (when Akiyami C leptospira is not contained)

### 5.2 Information to be provided in package insert and other labeling

- (1) Caution that the product be rendered homogenous by thorough shaking before use
- (2) Recommended human dose and route of administration, as follows:

For primary immunization, generally 2 doses of 1mL are given by subcutaneous injection at an interval of 7 days. For booster immunization, generally 1mL is given by subcutaneous injection.

# WHOLE HUMAN BLOOD

## 1. Descriptive definition

“Whole Human Blood” is a deep-red liquid product containing human blood which has been mixed with anticoagulant solution and stored.

On standing it separates into a red sediment of erythrocytes and a yellow supernatant layer. A grayish layer composed mainly of leukocytes may form on the surface of the sediment. In the supernatant turbidity due to the presence of fat and slight color due to the presence of free hemoglobin may be observed. A blood sample (segment tube) shall be provided as a package unit.

## 2. Production control

### 2.1 Source material

#### 2.1.1 Anticoagulant solutions

|   | Solution A<br>(ACD-A) | Solution C<br>(CPD) |
|---|-----------------------|---------------------|
| Tri-sodium citrate                      | 22.0 g                | 26.30 g             |
| Citric acid                             | 8.0 g                 | 3.27 g              |
| Glucose                                 | 22.0 g                | 23.20 g             |
| Sodium dihydrogen phosphate (dihydrate) |                       | 2.51 g              |

To formula A or C, water for injection shall be added to make 1000 mL of anticoagulant solution. Anticoagulant solution shall be subjected to the tests given in 3.1.

A 15-mL portion of solution A or a 14-mL portion of solution C shall be used to 100 mL of blood.

#### 2.1.2 Human blood

Human blood shall be mixed with anticoagulant solution and kept at 2–6°C after collection.

When whole human blood is separated in respective components within 6 hours (Solution A) or 8 hours (Solution C) after collection, it can be kept at room temperature after separation.

### 2.2 Blood sample (segment tube) for laboratory tests

Blood taken into collecting tube or test tube closed at both ends shall serve as test sample.

## 3. Control tests

### 3.1 Tests on anticoagulant solution

Anticoagulant solution A shall be subjected to the tests 3.1.1, 3.1.2, 3.1.3, 3.1.4, 3.1.6 and 3.1.7, while solution C shall be subjected to all the tests given below.

#### 3.1.1 Test for pH

When the test given in General Tests is applied, the pH of the solutions A and C shall be within the range of 4.5 to 5.5 and 5.4 to 5.8, respectively.

#### 3.1.2 Test for citric acid content

When the test given in General Tests is applied, the citric acid content shall be  $0.80 \pm 0.04$  w/v% in solution A and  $0.32 \pm 0.02$  w/v% in solution C.

#### 3.1.3 Test for sodium citrate content

When the test given in General Tests is applied, the trisodium citrate content shall be  $2.20 \pm 0.11$  w/v% in solution A and  $2.63 \pm 0.13$  w/v% in solution C.

#### 3.1.4 Test for glucose content

When the test given in General Tests is applied, the glucose content shall be  $2.20 \pm 0.11$  w/v% in solution A and  $2.32 \pm 0.12$  w/v% in solution C.

#### 3.1.5 Test for sodium dihydrogen phosphate content

When the test given in General Tests is applied, the sodium dihydrogen phosphate (dihydrate) content shall be  $0.25 \pm 0.01$  w/v%.

#### 3.1.6 Sterility test

The test given in General Tests shall apply.

#### 3.1.7 Pyrogen test

The test given in General Tests or the bacterial endotoxins test given in General Tests, Processes and Apparatus in the JP shall apply. Test sample shall be prepared by adding 100 mL of isotonic sodium chloride solution to 15 mL of solution A or to 14 mL of solution C. When the bacterial endotoxins test is applied, the content of endotoxin shall not be over 0.5 EU/mL.

### 3.2 Tests on final product

#### 3.2.1 Inspection

Whole Human Blood, on visual inspection, shall be free from marked hemolysis, coagulation, and changes in color or other abnormal findings.

#### 3.3.2 Sterility test

In order to check aseptic procedures in production processes, at least one container per 100 shall comply with the test given in General Tests.

In this case, the total volume of inoculum required per container shall be 10 mL, the number of vessels required per container shall be 2 and the volume of inoculum per vessel shall be 5 mL.

For this test, out-of-date blood or blood which has not been used as Whole Human Blood because of non-conformity to the test given in 3.2.2 or of any other reason may also be used.

## 4. Storage and expiry date

Whole Human Blood shall be stored at a temperature of 2–6°C. The approved expiry date after the date of collection of blood shall be applied.

## 5. Other requirements

### 5.1 Labeling contents

- (1) Date on which the blood was collected
- (2) ABO group and positivity or negativity for D(Rho) antigen
- (3) The product shall not be used for transfusion if there is any abnormality on visual inspection.
- (4) The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

### 5.2 Labeling on blood sample (segment tube) for laboratory tests

The following information may be provided on the direct container if integral segments of donor tubing are used.

- (1) Production number of the Whole Human Blood
- (2) Name of anticoagulant solution, if used

5.3 Information to be provided in package insert and other labeling

- (1) Name and amount or ratio of anticoagulant solution in the blood sample (segment tube) for laboratory tests
- (2) The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

## **CONCENTRATED HUMAN RED BLOOD CELLS**

### **1. Descriptive definition**

“Concentrated Human Red Blood Cells” is a dark red liquid product containing human red blood cells, prepared by removing plasma and most of the white blood cells from whole human blood, and mixing with an appropriate red blood cell storage solution. On standing it separates into a sediment mainly composed of erythrocytes and a yellow supernatant fluid, in which coloring due to the presence of hemoglobin may be observed.

A blood sample (segment tube) for laboratory test shall be provided as a package unit.

### **2. Production control**

#### **2.1 Source material**

Whole Human Blood given in 2.1.2 shall be used.

#### **2.2 Processing**

Plasma shall be separated within 24 hours after the collection of blood.

#### **2.3 Blood sample (segment tube) for laboratory tests**

Requirements given in 2.2 of Whole Human Blood shall apply.

### **3. Control tests**

Requirements given in 3.2 of Whole Human Blood shall apply.

### **4. Storage and expiry date**

Concentrated Human Red Blood Cells shall be stored at 2–6°C. The approved expiry date after the date of collection of blood shall be applied.

### **5. Other requirements**

#### **5.1 Labeling contents**

- (1) Date on which the blood was collected
- (2) ABO blood group and positivity or negativity for D(Rho) antigen
- (3) Components and amount of a preservative used for the storage of red blood cells
- (4) The product shall not be used if there is any abnormality on visual inspection.
- (5) The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

#### **5.2 Labeling on blood sample (segment tube) for laboratory tests**

The following information may be provided on the direct container if integral segments of donor tubing are used.

- (1) Production number of the Concentrated Human Red Blood Cells
- (2) Name of anticoagulant solution, if used

#### **5.3 Information to be provided in package insert and other labeling**

- (1) Name and amount or ratio of anticoagulant solution in the blood sample (segment tube) for laboratory tests
- (2) The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

## **WASHED HUMAN RED BLOOD CELLS**

### **1. Descriptive definition**

“Washed Human Red Blood Cells” is a dark red liquid product containing human red blood cells, prepared by suspending washed sediment of “Concentrated Human Red Blood Cells” in isotonic sodium chloride.

On standing, it separates into a sediment composed mainly of erythrocytes and a clear fluid layer where slight coloring due to the presence of hemoglobin may be observed.

A blood sample (segment tube) for laboratory tests shall be provided as a package unit.

### **2. Production control**

#### **2.1 Source material**

'Concentrated Human Red Blood Cells' shall be used.

#### **2.2 Processing**

Sediment of 'Concentrated Human Red Blood Cells' shall be washed and resuspended in isotonic sodium chloride solution within 10 days after the collection of blood.

#### **2.3 Blood sample (segment tube) for laboratory tests**

The blood sample (segment tube) provided as a package unit shall serve as the test sample for the product.

### **3. Control tests**

Requirements given in 3.2 of Whole Human Blood shall apply.

### **4. Storage and expiry date**

Washed Human Red Blood Cells shall be stored at 2–6°C. The expiry date shall be 24 hours.

### **5. Other requirements**

#### **5.1 Labeling contents**

- (1) Date on which blood was collected;
- (2) ABO blood group and positivity or negativity for D(Rho) antigen
- (3) The product shall not be used for transfusion if there is any abnormality on visual inspection.
- (4) The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

#### **5.2 Labeling on blood sample (segment tube) for laboratory tests**

The following information may be provided on the direct container if integral / ? contiguous segments of donor tubing are used.

- (1) Production number of the Washed Human Red Blood Cells

#### **5.3 Information to be provided in package insert and other labeling**

The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

## **LEUKOCYTE-POOR RED BLOOD CELLS**

### **1. Descriptive definition**

“Leukocyte-poor Red Blood Cells” is a dark red liquid product prepared by suspending washed “Concentrated Human Red Blood Cells” from which most of leukocytes have been removed in isotonic sodium chloride solution.

On standing, it separates into a sediment composed mainly of erythrocytes and a clear fluid layer where slight coloring due to the presence of hemoglobin may be observed.

A blood sample (segment tube) for laboratory tests shall be provided as a package unit.

### **2. Production control**

#### **2.1 Source material**

'Concentrated Human Red Blood Cells' shall be used.

#### **2.2 Processing**

Sediment of "Concentrated Human Red Blood Cells" shall be resuspended in isotonic sodium chloride solution after leukocytes are removed by filtration within 10 days after the collection of blood.

#### **2.3 Blood sample (segment tube) for laboratory tests**

After washing, red blood cells collected into a pilot tube filled with red blood cells shall serve as the pilot sample of blood for compatibility tests.

### **3. Control tests**

#### **3.1 Inspection**

Requirements given in 3.2.1 of Whole Human Blood shall apply.

#### **3.2 Test on the rate of elimination of leukocytes**

Manufacturers producing more than 1,000 containers per year shall ensure the compliance of at least 5 containers per 6 months. Those producing less than 1,000 but more than 100 containers per year shall ensure the compliance of 1 container per 100, and those producing less than 100 containers per year shall ensure the compliance of 1 container per year for the test.

When counted in a counting chamber or with an automated counter, more than 90% of leukocytes shall be eliminated from the original blood.

#### **3.3 Sterility test**

Requirements given in 3.2.2 of Whole Human Blood shall apply.

### **4. Storage and expiry date**

Leukocyte-poor Red Blood Cells shall be stored at 2–6°C. The expiry date shall be 24 hours.

### **5. Other requirements**

#### **5.1 Labeling contents**

- (1) Date on which the blood was collected
- (2) ABO blood group and positivity or negativity for D(Rho) antigen.
- (3) Name and amount of red blood cell storage solution, if the product is not washed
- (4) The product shall not be used if there is any abnormality on visual inspection.

(5) The transfusion set specified in Item 45 of General Rules for Blood product shall be used.

5.2 Labeling on blood sample (segment tube) for laboratory tests

The following information may be provided on the direct container if integral / ? contiguous segments of donor tubing are used.

(1) Production number of the Leukocyte Poor Red Blood Cells

(2) Name of anticoagulant solution and red blood cell storage solution, if the product is not washed.

5.3 Information to be provided in package insert and other labeling

The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

## **FROZEN-THAWED HUMAN RED BLOOD CELLS**

### **1. Descriptive definition**

“Concentrated Frozen-thawed Human Red Cells” is a dark red liquid product prepared by thawing “Concentrated Human Red Blood Cells” that have been stored in a frozen state in the presence of a suitable cryoprotective substance which is subsequently removed by a washing process.

A blood sample (segment tube) shall be provided as a package unit.

### **2. Production control**

#### **2.1 Source material**

Concentrated Human Red Blood Cells shall be used.

#### **2.2 Freezing**

Plasma shall be removed within 5 days after the collection of blood. Glycerol or other suitable cryoprotective substance shall be added to the red cells which shall be kept frozen at -65°C or below.

The period of storage in the frozen state shall be less than 10 years.

#### **2.3 Thawing and washing**

Frozen red cells shall be thawed at a temperature not higher than 40°C and the cryoprotective substance shall be removed by centrifugation or other suitable washing method.

#### **2.4 Blood sample (segment tube) for laboratory tests**

After washing, a portion of red cells collected shall be placed into a tube and serve as pilot sample of blood for compatibility tests.

### **3. Control tests**

#### **3.1 Inspection**

Requirements given in 3.2.1 of Whole Human Blood shall apply.

#### **3.2 Weight**

When weighed by a suitable method, not less than 63 g of red cells shall be recovered from 200 mL of source material.

#### **3.3 Test for hemoglobin content**

When the test for Hemoglobin Content given in General Tests is applied, 1mL of the final product shall contain not less than 0.24 g of hemoglobin.

#### **3.4 Sterility test**

The test given in 3.2.2 of Whole Human Blood shall apply.

### **4. Storage and expiry date**

Concentrated Frozen-thawed Human Red Cells shall be stored at 2–6°C. The expiry date shall be 12 hours.

### **5. Other requirements**

#### **5.1 Labeling contents**

- (1) Date on which the blood was collected
- (2) Production number of the source material
- (3) ABO group and positivity or negativity for D(Rho) antigen

- (4) Date and hour on which the red cells were thawed
  - (5) The product shall not be used if there is any abnormality on visual inspection.
  - (6) The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.
- 5.2 Labeling on blood sample (segment tube) for laboratory tests
- The following information may be provided on the direct container if integral / ? contiguous segments of donor tubing are used.
- (1) Production number of the Concentrated Frozen-thawed Human Red Cells
- 5.3 Information to be provided in package insert and other labeling
- (1) Components and amount or ratio of the solution used in the final washing step.
  - (2) The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

## **FRESH-FROZEN HUMAN PLASMA**

### **1. Descriptive definition**

“Fresh-frozen Human Plasma” is a product containing human blood plasma prepared from the blood of a single individual, and stored in a frozen state with a minimum loss of activity of various clotting factors. When thawed, it becomes a yellowish or yellowish-brown liquid which may be turbid due to the presence of fat.

A sample for laboratory testing shall be provided as a package unit.

### **2. Production control**

#### **2.1 Source material**

Either human blood type listed below shall be used.

- (1) Whole Human Blood according to 2.1.2
- (2) Plasma or Platelet-rich Plasma collected by hemapheresis.

#### **2.2 Separation and freezing of plasma**

Within 6 hours (Solution A) or 8 hours (Solution C) after the collection of blood, plasma shall be separated and frozen at -20°C or less.

A sealed segment of integral donor tubing, filled with plasma, shall serve as test sample.

#### **2.3 Blood sample (segment tube) for laboratory tests**

A portion of plasma collected in a pilot tube shall serve as the blood sample for compatibility tests.

### **3. Control tests**

At least one container per 500 shall be subjected to the tests given in 3.2 and 3.3. For the tests, out-of-date plasma or blood which has not been used as a product because of non-conformity with the serological test for syphilis may also be used as test materials.

#### **3.1 Inspection**

Fresh-frozen Human Plasma, upon visual inspection, shall be free from marked hemolysis, change in color or other abnormal findings.

#### **3.2 Coagulation test**

To 0.1 mL of the test material, taken in a test tube kept in a water-bath at 37°C, 0.1 mL of thromboplastin solution and 0.1 mL of 0.025 M calcium chloride solution shall be added, and the time until the formation of a fibrin clot shall be recorded, and shall be within 20 seconds.

#### **3.3 Sterility test**

The test given in General Tests for bacterial and mycotic sterility shall apply, excluding the following, namely the requirements in Table 1 that the volume of inoculum per container be 10 mL and that the number of vessels required per container be 2. Two vessels containing the medium shall be inoculated with 5mL each.

### **4. Storage and expiry date**

Fresh-frozen Human Plasma shall be stored at -20°C or less.

The expiry date shall be one year after the collection of blood.

## **5. Other requirements**

### 5.1 Labeling contents

- (1) Date on which the blood was collected;
- (2) ABO blood group and positivity or negativity for D(Rho) antigen of the source material
- (3) The product shall be used within 3 hours after it is thawed.
- (4) The product shall not be used if there is any abnormality on visual inspection.
- (5) The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

### 5.2 Labeling on pilot sample of plasma

The following information may be provided on the direct container.

- (1) Production number of Fresh-frozen Human Plasma
- (2) Name of anticoagulant solution, if used

### 5.3 Information to be provided in package insert and other labeling

The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

## **PLATELET CONCENTRATE**

### **1. Descriptive definition**

“Platelet Concentrate” is a product containing human blood plasma prepared from the blood of an individual. It is a yellowish or yellowish-brown liquid which may be turbid due to the presence of fat.

A sample for laboratory testing (segment tube) shall be provided as a package unit.

### **2. Production control**

#### 2.1 Source material

Either human blood type listed below shall be used.

- (1) Whole Human Blood according to 2.1.2
- (2) Platelet-rich Plasma or concentrated Platelet Plasma by hemapheresis

#### 2.2 Separation of platelets

Platelets shall be suspended in plasma by centrifugation or other appropriate method.

#### 2.3 Blood sample (segment tube) for laboratory tests

A sealed segment of integral donor tubing, filled with plasma, shall serve as test sample.

### **3. Control tests**

Randomly selected samples shall be subjected to the tests given in 3.2 and 3.3. At least one container per 500 shall be subjected to the tests given in 3.4. For the tests, out-of-date plasma or plasma which has not been used as a product because of non-conformity to the serological test for syphilis may also be used as test materials.

#### 3.1 Inspection

Platelet, upon visual inspection, shall be free from marked hemolysis, change in color or other abnormal findings.

#### 3.2 Platelet count test

The final product shall have a platelet count of not less than  $0.2 \times 10^{11}$  of Platelet count

#### 3.3 Red blood cell count and white blood cell count tests

The final product shall contain normal counts of red blood cells and white blood cells.

#### 3.4 Sterility test

Requirements given in 3.2.2 of Whole Human Blood shall apply. At least one container per 500 shall be subjected to the test.

### **4. Storage and expiry date**

Platelet concentrate shall be stored at 20–24°C with shaking. The approved expiry date shall be applied.

### **5. Other requirements**

#### 5.1 Labeling contents

- (1) Date on which the blood was collected

- (2) ABO blood group and positivity or negativity for D(Rho) antigen of the source material
- (3) The product shall not be used if there is any abnormality on visual inspection.
- (4) The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

5.2 Labeling on pilot sample of plasma

The following information may be provided on the direct container.

- (1) Production number of the Platelet Concentrate
  - (2) Name of anticoagulant solution, if used
- 5.3 Information to be provided in package insert and other labeling

The transfusion set specified in Item 45 of General Rules for Blood Products shall be used.

## HUMAN PLASMA PROTEIN FRACTION

### 1. Descriptive definition

“Human Plasma Protein Fraction” is a clear, amber-colored liquid product containing albumin and certain globulin fractions which retain their solubility on heating.

### 2. Production control

#### 2.1 Source material

Requirements given in 1-4 of General Notice and 2-2-(6), (7) in General Rules for Blood Products shall apply.

#### 2.2 Fractionation

Albumin serving as bulk material shall be prepared by fractionating pooled source plasma using a method shown not to cause the deterioration of albumin and other plasma protein fractions.

#### 2.3 Final bulk and heat treatment

The final bulk shall be prepared by dissolving the bulk material with suitable stabilizing and isotonicizing agents and then distributing it into final containers. Albumin concentration shall be no less than 4.4 w/v%.

Immediately after filling, final containers shall be heated at  $60.0 \pm 0.5^{\circ}\text{C}$  for more than 10 hours.

### 3. Tests on final product

Following tests shall apply to each final lot.

#### 3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.4.

#### 3.2 Test for potassium content

When the test given in General Tests is applied, the potassium content shall be no more than 0.1 mg/mL.

#### 3.3 Test for sodium content

When the test given in General Tests is applied, the sodium content shall be no more than 3.7 mg/mL.

#### 3.4 Test for chloride content

The test given in the General Tests shall apply.

#### 3.5 Test for heme content

The test given in General Tests shall apply to a test sample prepared by dilution with water for injection to make an albumin concentration of 1 w/v%.

#### 3.6 Test for albumin content

When the test for protein nitrogen content and electrophoretic test given in General Tests are applied, the albumin content calculated from the results shall be within 90–110% of the value stated on the label. Furthermore, albumin shall consist of not less than 80% of total proteins, nor shall the gamma-globulin fraction significantly exceed 1%.

#### 3.7 Immunological identification test

A distinct precipitation line shall be formed in albumin region by immunoelectrophoresis against antiserum to human serum, and no unspecific lines of precipitation shall be observed.

#### 3.8 Sterility test

The test given in General Tests shall apply.

3.9 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.10 Pyrogen test

Either the pyrogen test or bacterial endotoxins test given in General Testing Methods shall apply. The content of bacterial endotoxins shall be no more than 0.2 EU/mL. When the content of bacterial endotoxins exceeds the criteria, the pyrogen test shall apply.

**4. Storage and expiry date**

Human Plasma Protein Fraction shall be stored at room temperature.

The expiry date shall be two years.

**5. Other requirements**

5.1 Labeling contents

(1) Albumin content per mL

(2) Sodium content per mL

(3) Chloride content per mL

5.2 Information to be provided in package insert and other labeling

The product shall not be used if it is found turbid.

## HUMAN SERUM ALBUMIN

### 1. Descriptive definition

“Human Serum Albumin” is a clear, green-yellow to yellow or amber colored liquid product containing serum albumin of human origin.

### 2. Production control

#### 2.1 Source material

Requirements given in 1-4 of General Notices and 2-2-(6), (7) in General Rules for Blood Products shall apply.

#### 2.2 Fractionation

Albumin serving as bulk material shall be prepared by fractionating pooled source plasma using a method shown not to cause the deterioration of albumin and other plasma fractions.

#### 2.3 Final bulk and heat-treatment

The final bulk shall be prepared by dissolving the bulk material with suitable stabilizing and isotonicizing agents. Immediately after filling, final containers shall be heated at  $60 \pm 0.5^{\circ}\text{C}$  for more than 10 hours. The albumin concentration shall be either 5 w/v% or 20–25 w/v%.

### 3. Tests on final product

Following tests shall apply to each final lot.

#### 3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.4.

#### 3.2 Test for sodium content

When the test given in General Tests is applied, the sodium content shall be no more than 3.7 mg/mL.

#### 3.3 Test for chloride content

When the test given in the General Tests is applied, the chloride content shall be within 90–110% of the value stated on the label.

#### 3.4 Test for heme content

The test given in General Tests shall apply to a test sample prepared by dilution with water for injection to make albumin concentration of 1 w/v%.

#### 3.5 Test for albumin content

The test given in General Tests shall apply. When the cellulose acetate membrane electrophoretic test in General Tests are applied, no less than 96% of total proteins shall have mobility of serum albumin, and the albumin content shall be within 90–110% of the value stated on the label.

#### 3.6 Immunological identification test

A distinct precipitation line shall be formed in the albumin region by immunoelectrophoresis against antiserum to human serum, and no unspecific lines of precipitation shall be observed.

#### 3.7 Sterility test

The test given in General Tests shall apply.

#### 3.8 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.9 Pyrogen test

Either the pyrogen test or bacterial endotoxins test given in General Testing Methods shall apply. The content of bacterial endotoxins shall be no more than 0.6 EU/mL. When the content of bacterial endotoxins exceeds the criteria, the pyrogen test shall apply.

## 4. Storage and expiry date

Hunan Serum Albumin shall be stored at room temperature.

The expiry date shall be two years.

## 5. Other requirements

### 5.1 Labeling contents

(1) Albumin content per mL

(2) Sodium content per mL

(3) Chloride content per mL

### 5.2 Information to be provided in package insert and other labeling

The product shall be used only if it is clear and free from deposits.

## **FREEZE-DRIED HUMAN FIBRINOGEN**

### **1. Descriptive definition**

“Freeze-dried Human Fibrinogen” is a freeze-dried product containing fibrinogen fraction in human plasma. When reconstituted, it becomes an almost colorless, slightly turbid liquid product.

### **2. Production control**

#### **2.1 Source plasma**

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### **2.2 Bulk material**

Bulk material shall be prepared by fractionating source plasma using a method shown not to cause the deterioration of fibrinogen.

#### **2.3 Final bulk and final product**

Final bulk shall be prepared by dissolving the bulk material with a suitable diluent. The final bulk shall then be filled into final containers and freeze-dried.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### **3.2 Test for solubility**

The product shall be soluble within 30 minutes at 37°C in the accompanying reconstituent. The resulting solution shall be free of deposits on visual inspection and no gelatinous matter or deposit shall be formed on standing for 2 hours at 15–20°C

#### **3.3 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.0 to 7.3.

#### **3.4 Tests for clottable protein content and purity**

For determination of total protein and clottable protein contents per mL of the sample, the test for protein nitrogen content given in General Tests shall apply. Clot obtained at 20–30°C by treating the test sample with sufficient amount of thrombin and calcium in the pH range of 6.6 to 7.4 shall be, after being thoroughly washed with suitable liquid, used for the determination of clottable protein. In these determinations the obtained values of protein nitrogen shall be converted to protein content by multiplying by 6.0 instead of 6.25.

No less than 50% of total proteins shall be clottable. In addition, the clottable protein content shall be no lower than 10 mg/mL and within 80–125% of the value stated on the label.

#### **3.5 Test for sodium citrate content**

When the test given in General Tests is applied, the sodium citrate content shall be no more than 700 mg/g of clottable protein.

#### **3.6 Sterility test**

The test given in General Tests shall apply.

#### **3.7 Test for freedom from abnormal toxicity**

The test given in General Tests shall apply.

### 3.8 Pyrogen test

The test given in General Tests shall apply. The dose of application is 2.5 mL/kg body weight.

### 3.9 Potency test

The sample shall be prepared by adding the appropriate buffer to the product so that the clottable protein concentration shall be 1 w/v%. When 0.9 mL of the test sample is added with 0.1 mL of isotonic sodium chloride solution containing 10 units per mL of thrombin, a firm clot shall be formed within 60 seconds, when the test is conducted at 20–30°C and kept constant within  $\pm 1^\circ\text{C}$ .

## **4. Storage and expiry date**

The expiry date shall be three years.

## **5. Other requirements**

### 5.1 Labeling contents

#### (1) Clottable protein content

(2) The transfusion set specified in Item 45 of General Rules for Blood Products shall be used and the product shall be administered within 1 hour of reconstitution.

#### (3) Sodium citrate content

### 5.2 Information to be provided in package insert and other labeling

(1) The product shall not be used if the reconstituted solution contains deposits.

(2) The transfusion set specified in Item 45 of General Rules for Blood Products shall be used and the product shall be administered within 1 hour of reconstituted.

### 5.3 Reconstituent

The reconstituent shall be water for injection.

## **FREEZE-DRIED HUMAN BLOOD COAGULATION FACTOR VIII**

### **1. Descriptive definition**

“Freeze-dried Human Blood Coagulation Factor VIII” is a freeze-dried product containing blood coagulation Factor VIII in human plasma (hereafter referred to as "Factor VIII"). When reconstituted, it becomes a pale yellow or yellow, turbid liquid.

### **2. Production control**

#### **2.1 Source plasma**

Usually, source plasma is prepared by pooling plasma from no more than two donors.

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### **2.2 Fractionation and freeze drying**

A fraction containing Factor VIII shall be prepared by fractionating source plasma using a method shown not to cause the deterioration of Factor VIII. An aliquot of the fraction diluted with an appropriate diluent shall serve as test sample, and the remaining fraction shall be filled into final containers and freeze-dried.

The test sample shall be subjected to the test given in 3.1.

### **3. Control tests**

#### **3.1 Control test on the test sample**

The test sample shall be diluted with an appropriate diluent to the concentration equal to that of the reconstituted final product and subjected to the test given in 3.2.4.

#### **3.2 Tests on final product**

The following tests shall be conducted on each group of the final product dried under separate conditions or at least one container per 100.

##### **3.2.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

##### **3.2.2 Test for protein content**

When the test for protein nitrogen content given in General Tests is applied, the protein content shall be no more than 20 mg per unit.

##### **3.2.3 Sterility test**

The test given in General Tests shall apply.

##### **3.2.4 Potency test**

A series of two-fold dilutions of the test sample and the international standard for Factor VIII or its reference shall be prepared using an appropriate buffer containing human serum albumin. A 0.1-mL portion of diluted sample or standard solution is precisely taken into a test tube, and 0.1 mL of human Factor VIII-deficient plasma and then 0.1 mL of APTT reagent shall be added to the tube and gently mixed. Test tubes shall be activated at 36.5–37.5°C, and 0.1 mL of 0.025 mol/L calcium chloride solution shall be added to determine the coagulation time. Factor VIII content calculated from test results shall be no less than 2 units and no less than 80% of the value stated on the label.

**4. Storage and expiry date**

The expiry date shall be one year.

**5. Other requirements**

5.1 Labeling contents

- (1) Factor VIII content per mL of the reconstituted product
- (2) The product shall be used within 1 hour after reconstitution.

5.2 Reconstituent

The reconstituent shall be either water for injection or physiological saline.

## **FREEZE-DRIED CONCENTRATED BLOOD COAGULATION FACTOR VIII**

### **1. Descriptive definition**

“Freeze-dried Concentrated Human Blood Coagulation Factor VIII” is a freeze-dried product containing blood coagulation Factor VIII of human plasma (hereafter referred to as "Factor VIII"), in which the content of clottable and other contaminating proteins is reduced. When reconstituted, it becomes a colorless or pale yellow, clear or slightly turbid liquid.

### **2. Production control**

#### **2.1 Source plasma**

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### **2.2 Bulk material**

The fraction containing Factor VIII shall be prepared by fractionating source plasma using a method shown not to cause the deterioration of Factor VIII. The fraction obtained shall serve as bulk material.

#### **2.3 Final bulk and final product**

The final bulk shall be prepared by dissolving the bulk material with a suitable diluent and filled into final containers and freeze-dried.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.5 to 8.0.

#### **3.3 Test for protein content**

When the test for protein nitrogen content given in General Tests is applied, the protein content shall be no more than 5 mg per unit.

#### **3.4 Test for clottable protein content**

When the test for clottable protein content and purity given in 3.4 of Freeze-dried Human Fibrinogen is applied, the clottable protein content shall be no more than 2 mg per unit.

#### **3.5 Sterility test**

The test given in General Tests shall apply.

#### **3.6 Test for freedom from abnormal toxicity**

The test given in General Tests shall apply.

#### **3.7 Pyrogen test**

The test given in General Tests shall apply. The dose of application is 10 units/kg body weight.

#### **3.8 Potency test**

A series of two-fold dilutions of the test sample and the international standard for Factor VIII or its reference shall be prepared using an appropriate buffer containing human serum albumin. A 0.1-mL portion of diluted sample or standard solution is precisely taken into a test tube, and 0.1 mL

of human Factor VIII-deficient plasma and then 0.1 mL of APTT reagent shall be added to the tube and gently mixed. Test tubes shall be activated at 36.5–37.5°C, and 0.1 mL of 0.025 mol/L calcium chloride solution shall be added to determine the coagulation time. Factor VIII content calculated from the test results shall be no less than 10 units and no less than 80% of the value stated on the label.

**4. Storage and expiry date**

The expiry date shall be two years.

**5. Other requirements**

5.1 Labeling contents

- (1) Factor VIII content per mL of the reconstituted product;
- (2) The product shall be used within 1 hour after reconstitution.

5.2 Reconstituent

The reconstituent shall be either water for injection or physiological saline.

# **FREEZE-DRIED HUMAN BLOOD COAGULATION FACTOR IX COMPLEX**

## **1. Descriptive definition**

“Freeze-dried Human Blood Coagulation Factor IX Complex” is a freeze-dried product containing blood coagulation Factor IX complex (hereafter referred to as "Factor IX") in human plasma. When reconstituted, it becomes a colorless or pale yellow, nearly clear liquid.

## **2. Production control**

### **2.1 Source plasma**

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

### **2.2 Bulk material**

The fraction containing Factor IX complex shall be obtained by fractionating source plasma using a method shown not to cause the deterioration of blood coagulation factors and other plasma proteins. The fraction shall serve as bulk material.

### **2.3 Final bulk and final product**

The final bulk shall be prepared by diluting the bulk material with a suitable diluent and filled into final containers and freeze-dried.

## **3. Tests on final product**

Following tests shall apply to each final lot.

### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.4.

### **3.3 Test for protein content**

When the test for protein nitrogen content given in General Tests is applied, the protein content shall be no more than 50 mg/mL.

### **3.4 Test for freedom from active coagulation factors**

A 0.4-mL portion of the test sample and 0.4 mL of 1% fibrinogen solution shall be mixed in a test tube (12×105 mm) and stirred in a water bath at 37°C. The coagulation test shall be started upon the placement of the tube containing the mixture in the water bath, and continued until coagulation occurs by observing coagulation time as fibrinogen coagulation time at approximately 15-minute intervals. The time until coagulation shall be no less than 2 hours.

### **3.5 Sterility test**

The test given in General Tests shall apply.

### **3.6 Test for freedom from abnormal toxicity**

The test given in General Tests shall apply.

### **3.7 Pyrogen test**

The test given in General Tests shall apply. The dose of application is 50 units/kg body weight.

### **3.8 Potency test**

A series of two-fold dilutions of the test sample and the international standard for Factor IX or

its reference shall be prepared using an appropriate buffer containing human serum albumin. A 0.1-mL portion of diluted sample or standard solution is precisely taken into a test tube, and 0.1 mL of human Factor IX-deficient plasma and then 0.1 mL of APTT reagent shall be added to the tube and gently mixed. Test tubes shall be activated at 36.5–37.5°C, and 0.1 mL of 0.025 mol/L calcium chloride solution shall be added to determine the coagulation time. Factor IX content calculated from test results shall be no less than 10 units and no less than 80% of the value stated on the label.

**4. Storage and expiry date**

The expiry date shall be two years.

**5. Other requirements**

5.1 Labeling contents

- (1) Factor IX content per mL of the reconstituted product
- (2) The product shall be used within 1 hour after reconstitution.

5.2 Reconstituent

The reconstituent shall be either water for injection or physiological saline.

# **FREEZE-DRIED CONCENTRATED HUMAN BLOOD COAGULATION FACTOR IX**

## **1. Descriptive definition**

“Freeze-dried Concentrated Human Blood Coagulation Factor IX” is a freeze-dried product containing blood coagulation Factor IX (hereafter referred to as "Factor IX") in human plasma. When reconstituted, it becomes a colorless or pale yellow, nearly clear liquid.

## **2. Production control**

### **2.1 Source plasma**

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

### **2.2 Bulk material**

The fraction containing Factor IX shall be obtained by fractionating source plasma using a method shown not to cause the deterioration of blood coagulation factors. The fraction shall serve as bulk material.

### **2.3 Final bulk and final product**

The final bulk shall be prepared by diluting the bulk material with a suitable diluent and filled into final containers and freeze-dried.

## **3. Tests on final product**

Following tests shall apply to each final lot.

### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.4.

### **3.3 Test for protein content**

When the test for protein nitrogen content given in General Tests is applied, the protein content shall be no more than 50 mg/mL.

### **3.4 Test for freedom from active coagulation factors**

The test given in 3.4 of Freeze-dried Human Blood Coagulation Factor IX Complex shall apply.

### **3.5 Sterility test**

The test given in General Tests shall apply.

### **3.6 Test for freedom from abnormal toxicity**

The test given in General Tests shall apply.

### **3.7 Pyrogen test**

The test given in General Tests shall apply. The dose of application is 50 units/kg body weight.

### **3.8 Potency test**

The test given in 3.8 of Freeze-dried Human Blood Coagulation Factor IX Complex shall apply.

### **3.9 Test for freedom from blood coagulation Factors II, VII and X**

For the measurement of coagulation time with human blood coagulation Factor II, a series of

two-fold dilutions of the product and normal human plasma pooled from no less than 10 donors shall be prepared using an appropriate buffer. A 0.1-mL portion of the diluted product sample or pooled plasma shall be added to 0.1 mL of human Factor II-deficient plasma in a test tube and mixed at 37°C until sufficient activation is achieved. Then, 0.2 mL of tissue thromboplastin solution containing 0.0125 mol/L calcium chloride solution shall be added to determine the coagulation time.

The measurement of coagulation time with human blood coagulation Factor VII shall be conducted in the same manner as described above, except for the use of human Factor VII-deficient plasma instead of human Factor II-deficient plasma.

The measurement of coagulation time with human blood coagulation Factor X shall be conducted in the same manner as described above, except for the use of human Factor X-deficient plasma instead of human Factor II-deficient plasma.

Factor II, VII and X content calculated from test results shall be no more than 0.01-fold each of normal human plasma per unit of Factor IX.

#### **4. Storage and expiry date**

The expiry date shall be two years.

#### **5. Other requirements**

##### 5.1 Labeling contents

- (1) Factor IX content per mL of the reconstituted product
- (2) The product shall be used within 1 hour after reconstitution.

##### 5.2 Reconstituent

The reconstituent shall be either water for injection or physiological saline.

## NORMAL HUMAN IMMUNOGLOBULIN

### 1. Descriptive definition

“Normal Human Immunoglobulin” is a clear, colorless or yellowish-brown liquid product containing immunoglobulin G of normal human serum.

### 2. Production control

#### 2.1 Source material

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### 2.2 Bulk material

The fraction containing immunoglobulin G shall be prepared by fractionating source plasma using a suitable method shown not to cause the deterioration of antibodies. The material thereby obtained shall serve as bulk material.

#### 2.3 Final bulk and final product

Suitable stabilizing and isotonicizing agents may be added to the bulk material to produce final bulk, which shall contain human immunoglobulin G at a concentration of not less than 10 w/v%. Suitable preservatives can be added in the final bulk.

### 3. Tests on final product

Following tests shall apply to each final lot, except for the test given in 3.2 if no preservative is used.

#### 3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

#### 3.2 Test for thimerosal content

When the test given in General Tests is applied, the thimerosal content shall be no more than 0.012 w/v%.

#### 3.3 Test for immunoglobulin G content

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the total protein shall have the mobility of normal human immunoglobulin G. In addition, when the test for protein nitrogen content given in General Tests is applied, the immunoglobulin G content per mL calculated from total protein content shall be within 90–110% of the value stated on the label.

#### 3.4 Immunological identification test

When tested by immunoelectrophoresis against antiserum to human serum, the final product shall form a distinct precipitation line of normal human immunoglobulin G and no unspecific lines of precipitation shall be observed.

#### 3.5 Sterility test

The test given in General Tests shall apply.

#### 3.6 Test for freedom from abnormal toxicity

The tests given in General Tests shall apply.

### 3.7 Pyrogen test

The test given in General Tests shall apply. The dose of application is 1.0 mL/kg body weight.

### 3.8 Titration test for measles

The titer shall be no less than 5 units per 150 mg of human immunoglobulin G, complying with the titration test for measles given in General Tests.

## **4. Storage and expiry date**

The expiry date shall be two years.

## **5. Other requirements**

### 5.1 Labeling contents

- (1) Immunoglobulin G content per mL
  - (2) Measles antibody content per mL
  - (3) The product shall not be injected intravenously.
  - (4) Name and amount of preservative, if used
- ### 5.2 Information to be provided in package insert and other labeling
- When preservative has not been used, this shall be stated.

## **REDUCED ALKYLATED NORMAL HUMAN IMMUNOGLOBULIN**

### **1. Descriptive definition**

“Reduced Alkylated Normal Human Immunoglobulin” is a clear, colorless or pale yellow liquid product containing reduced alkylated immunoglobulin G.

### **2. Production control**

#### **2.1 Source material**

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### **2.2 Bulk material**

Immunoglobulin G fraction shall be prepared by fractionating source plasma using a suitable method shown not to cause the deterioration of antibodies and to be free from a risk of transmission of hepatitis viruses or other microorganisms. The immunoglobulin G fraction shall be treated with suitable reducing and alkylating agents to reduce and alkylate interchain disulfide bonds in immunoglobulin G such that not more than 7% of the component shall have the mobility equivalent to that of normal human immunoglobulin G. After the treatment, the reducing and alkylating agents shall be removed, and the material thereby obtained shall serve as bulk material.

#### **2.3 Final bulk and final product**

Suitable stabilizing and isotonicizing agents may be added to the bulk material to produce final bulk, which shall contain human immunoglobulin G at a concentration of not less than 5 w/v%.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### **3.1 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

#### **3.2 Test for alkylated immunoglobulin G content**

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, not less than 90% of the proteins shall have the mobility of normal human immunoglobulin G. In addition, when the test for protein nitrogen content given in General Tests is applied, the alkylated immunoglobulin G content per mL calculated from total protein content shall be within 90–110% of the value stated on the label.

#### **3.3 Test for alkylation**

Test sample shall be prepared by heating the test material at 100°C for 2 minutes in 8 M urea solution containing 1 w/v% sodium dodecylsulfate. The test sample shall be subjected to disc electrophoresis in polyacrylamide gel containing sodium dodecylsulfate, using 0.1 M phosphate buffer (pH 7.2) containing 0.1 w/v% sodium dodecylsulfate. The alkylation of immunoglobulin G shall be confirmed by continuous determination of protein absorbance of the stained gel.

#### **3.4 Immunological identification test**

When tested by immunoelectrophoresis against antiserum to human serum, the final product shall form a distinct precipitation line of alkylated immunoglobulin G and no unspecific lines of precipitation shall be observed.

3.5 Sterility test

The test given in General Tests shall apply.

3.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.7 Pyrogen test

The test given in General Tests shall apply.

3.8 Titration test for measles

The titer shall be no less than 5 units per 150 mg of alkylated human immunoglobulin G, complying with the titration test for measles given in General Tests.

**4. Storage and expiry date**

The expiry date shall be two years.

**5. Other requirements**

5.1 Labeling contents

Alkylated human immunoglobulin G content per mL

5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains insoluble matters.

# **FREEZE-DRIED ION-EXCHANGE-RESIN-TREATED NORMAL HUMAN IMMUNOGLOBULIN**

## **1. Descriptive definition**

“Freeze-dried Ion-exchange-resin-treated Normal Human Immunoglobulin” is a freeze-dried product containing ion-exchange-resin-treated human immunoglobulin G. When reconstituted, it becomes a pale yellow, clear or slightly turbid liquid, and contains almost no visible deposits.

## **2. Production control**

### **2.1 Source material**

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

### **2.2 Bulk material**

Immunoglobulin G fraction shall be prepared by fractionating source plasma using a suitable method shown not to cause the deterioration of antibodies and to be free from a risk of the transmission of hepatitis viruses or other microorganisms. The immunoglobulin G fraction shall be purified by ion-exchange chromatography, and the material thereby obtained shall serve as bulk material.

### **2.3 Final bulk and final product**

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers and freeze-dried. When reconstituted by the method stated on the label, the product should contain human immunoglobulin G at a concentration of not less than 5 w/v%.

## **3. Tests on final product**

Following tests shall apply to each final lot.

### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

### **3.3 Test for immunoglobulin G content**

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the total protein, from which added human serum albumin is excluded, shall have the mobility of normal human immunoglobulin G. In addition, when the test for protein nitrogen content given in General Tests is applied, the immunoglobulin G content per mL calculated from total protein content shall be within 90–110% of the value stated on the label

### **3.4 Test for freedom from aggregated immunoglobulin G**

When gel permeation chromatography is conducted using a suitable supporting medium for the fractionation of immunoglobulin G, higher order aggregates more than dimer of immunoglobulin G shall be detected at a concentration of no more than 1.0%.

### 3.5 Immunological identification test

When tested by immunoelectrophoresis against antiserum to human serum, the final product shall form a distinct precipitation line of normal human immunoglobulin G and no unspecific line of precipitation shall be observed.

### 3.6 Test for freedom from anti-complementary effect

The test given in General Tests shall apply.

### 3.7 Sterility test

The test given in General Tests shall apply.

### 3.8 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.9 Pyrogen test

The test given in General Tests shall apply.

### 3.10 Titration test for measles

The titer shall be no less than 5 units per 150 mg of human immunoglobulin G, complying with the titration test for measles given in General Tests.

## **4. Storage and expiry date**

The expiry date shall be two years.

## **5. Other requirements**

### 5.1 Labeling contents

Human immunoglobulin G content per mL of the reconstituted product

### 5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituent solution contains marked precipitates.

### 5.3 Reconstituent

The reconstituent shall be water for injection

## **FREEZE-DRIED SULFONATED NORMAL HUMAN IMMUNOGLOBULIN**

### **1. Descriptive definition**

“Freeze-dried Sulfonated Normal Human Immunoglobulin” is a freeze-dried product containing sulfonated human immunoglobulin G. When reconstituted, it becomes a pale yellow clear or slightly turbid liquid, and contains almost no visible deposits.

### **2. Production control**

#### **2.1 Source material**

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### **2.2 Bulk material**

Immunoglobulin G fraction shall be prepared by fractionating source plasma using a suitable method shown not to cause the deterioration of antibodies and to be free from a risk of the transmission of hepatitis virus or other microorganisms. The immunoglobulin G fraction shall be treated with a suitable sulfonating agent to sulfonate interchain disulfide bonds in immunoglobulin G such that no more than 5% of the component shall have the mobility equivalent to normal human immunoglobulin G. After the treatment, the sulfonating agent shall be removed, and the material thereby obtained shall serve as bulk material.

#### **2.3 Final bulk and final product**

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers and freeze-dried. When reconstituted by the method stated on the label, the product should contain sulfonated human immunoglobulin G at a concentration of not less than 5 w/v%.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

#### **3.3 Test for immunoglobulin G content**

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the total protein, from which added human serum albumin is excluded, shall have the mobility of normal human immunoglobulin G. In addition, when the test for protein nitrogen content given in General Tests is applied, the immunoglobulin G content per mL calculated from total protein content shall be within 90–110% of the value stated on the label.

#### **3.4 Test for sulfonation**

Test sample shall be prepared by heating the test material at 100°C for 2 minutes in 8M urea solution containing 1 w/v% sodium dodecylsulfate and subjected to electrophoresis in polyacrylamide gel containing sodium dodecylsulfate, by using 0.1M phosphate buffer (pH 7.2)

containing 0.1 w/v% sodium dodecylsulfate. By the continuous determination of protein absorbance of the stained gel, the sulfonation of immunoglobulin G shall be confirmed.

#### 3.5 Immunological identification test

When tested by immunoelectrophoresis against antiserum to human serum, the final product shall form a distinct precipitation line of normal human immunoglobulin G and no other abnormal lines of precipitation shall be observed.

#### 3.6 Test for freedom from anticomplementary effect

The test given in General Tests shall apply.

#### 3.7 Sterility test

The test given in General Tests shall apply.

#### 3.8 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.9 Pyrogen test

The test given in General Tests shall apply.

#### 3.10 Titration test for measles

The titer shall be no less than 5 units per 150 mg of sulfonated human immunoglobulin G, complying with the titration test for measles given in General Tests.

### **4. Storage and expiry date**

The expiry date shall be two years.

### **5. Other requirements**

#### 5.1 Labeling contents

Sulfonated Human immunoglobulin G content per mL of the reconstituted product

#### 5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains insoluble matters.

#### 5.3 Reconstituent

The reconstituent shall be water for injection.

## **pH 4-TREATED NORMAL HUMAN IMMUNOGLOBULIN**

### **1. Descriptive definition**

“pH 4-treated Normal Human Immunoglobulin” is a colorless clear liquid product containing pH 4-treated human immunoglobulin G.

### **2. Production control**

#### 2.1 Source material

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### 2.2 Bulk material

Immunoglobulin G fraction shall be prepared by fractionating source plasma using a suitable method shown not to cause the deterioration of antibodies and to be free from a risk of transmission of hepatitis viruses or other microorganisms. The immunoglobulin G fraction shall be dialyzed and ultrafiltrated at pH 4. The material thereby obtained shall serve as bulk material.

#### 2.3 Final bulk and final product

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers. The product should contain human immunoglobulin G at a concentration of not less than 5 w/v%.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### 3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 3.2 to 4.2.

#### 3.2 Test for immunoglobulin G content

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, not less than **98%** of the total protein shall have the mobility of normal human immunoglobulin G. In addition, when the test for protein nitrogen content given in General Tests is applied, the immunoglobulin G content per mL calculated from total protein content shall be within 90–110% of the value stated on the label.

#### 3.3 Test for freedom from aggregated immunoglobulin G

When gel permeation chromatography is conducted using a suitable supporting medium for the fractionation of immunoglobulin G, higher order aggregates more than dimer of immunoglobulin G shall be detected at a concentration of no more than 1.0%.

#### 3.4 Immunological identification test

When tested by immunoelectrophoresis against antiserum to human serum, the final product shall form a distinct precipitation line of normal human immunoglobulin G and no unspecific lines of precipitation shall be observed.

#### 3.5 Sterility test

The test given in General Tests shall apply.

#### 3.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.7 Pyrogen test

The test given in General Tests shall apply.

3.8 Titration test for measles

The titer shall be no less than 5 units per 150 mg of human immunoglobulin G, complying with the titration test for measles given in General Tests.

**4. Storage and expiry date**

The expiry date shall be two years.

**5. Other requirements**

5.1 Labeling contents

Human immunoglobulin G content per mL of the reconstituted product

5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains insoluble matters.

## **FREEZE-DRIED pH 4-TREATED NORMAL HUMAN IMMUNOGLOBULIN**

### **1. Descriptive definition**

“Freeze-dried pH 4-treated Normal Human Immunoglobulin” is a freeze-dried product containing pH 4-treated human immunoglobulin G. When reconstituted, it becomes a colorless clear or slightly turbid liquid, and contains almost no visible deposits.

### **2. Production control**

#### 2.1 Source material

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### 2.2 Bulk material

Immunoglobulin G fraction shall be prepared by fractionating source plasma using a suitable method shown not to cause the deterioration of antibodies and to be free from a risk of transmission of hepatitis viruses or other microorganisms. The immunoglobulin G fraction shall be treated with a pH-4 solution containing a small amount of pepsin, and then neutralized. The material thereby obtained shall serve as bulk material.

#### 2.3 Final bulk and final product

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers and freeze-dried. The product should contain protein at a concentration of not less than 5 w/v%.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### 3.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### 3.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.2 to 7.0.

#### 3.3 Test for immunoglobulin G content

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the total protein shall have the mobility of normal human immunoglobulin G. In addition, when the test for protein nitrogen content given in General Tests is applied, the immunoglobulin G content per mL calculated from total protein content shall be within 90–110% of the value stated on the label.

#### 3.4 Test for freedom from aggregated immunoglobulin G

When gel permeation chromatography is conducted using a suitable supporting medium for the fractionation of immunoglobulin G, higher order aggregates more than dimer of immunoglobulin G shall be detected at a concentration of no more than 1.0%.

#### 3.5 Immunological identification test

When tested by immunoelectrophoresis against antiserum to human serum, the final product

shall form a distinct precipitation line of normal human immunoglobulin G and no unspecific lines of precipitation shall be observed.

3.6 Test for freedom from anticomplementary effect

The test given in General Tests shall apply.

3.7 Sterility test

The test given in General Tests shall apply.

3.8 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.9 Pyrogen test

The test given in General Tests shall apply.

3.10 Titration test for measles

The titer shall be no less than 5 units per 150 mg of human immunoglobulin G, complying with the titration test for measles given in General Tests.

**4. Storage and expiry date**

Storage temperature shall be kept at 20°C or less.

The expiry date shall be two years.

**5. Other requirements**

5.1 Labeling contents

Human immunoglobulin G content per mL of the reconstituted product

5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains marked precipitates.

5.3 Reconstituted

The reconstituent shall be physiological saline.

# **FREEZE-DRIED PLASMIN-TREATED NORMAL HUMAN IMMUNOGLOBULIN**

## **1. Descriptive definition**

“Freeze-dried Plasmin-treated Normal Human Immunoglobulin” is a freeze-dried product containing immunoglobulin G and plasmin-treated fragment of human immunoglobulin G. When reconstituted, it becomes a clear or slightly whitish turbid liquid, and contains almost no visible deposits.

## **2. Production control**

### **2.1 Source material**

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

### **2.2 Bulk material**

Immunoglobulin G fraction shall be prepared by fractionating source plasma using a method shown to be free from a risk of transmission of hepatitis viruses or other microorganisms and not to cause the deterioration of antibodies. The immunoglobulin G fraction shall be treated with plasmin using a method that could render the concentration of undigested immunoglobulin G to  $40 \pm 10\%$  of the total immunoglobulin G. The material thereby obtained shall serve as bulk material.

### **2.3 Final bulk and final product**

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers and freeze-dried. When reconstituted by the method stated on the label, the product should contain plasmin-treated human immunoglobulin G fragment at a concentration of not less than 5 w/v%.

## **3. Tests on final product**

Following tests shall apply to each final lot.

### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

### **3.3 Test for plasmin-treated immunoglobulin G content**

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the protein shall have the mobility of normal human immunoglobulin G. In addition, when the test for protein nitrogen content given in General Tests is applied, the plasmin-treated immunoglobulin G content per mL calculated from total protein content shall be within 90–110 % of the value stated on the label.

### **3.4 Immunological identification test**

When tested by immunoelectrophoresis against antiserum to human serum, the final product shall form a distinct precipitation line of plasmin-treated human immunoglobulin G and no

unspecific lines of precipitation shall be observed.

3.5 Test for freedom from anti-complementary effect

The test given in General Tests shall apply.

3.6 Sterility test

The test given in General Tests shall apply.

3.7 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.8 Pyrogen test

The test given in General Tests shall apply.

3.9 Titration test for measles

The titer shall be no less than 5 units per 150 mg of plasmin-treated human immunoglobulin G, complying with the titration test for measles given in General Tests.

**4. Storage and expiry date**

The expiry date shall be three years.

**5. Other requirements**

5.1 Labeling contents

Plasmin-treated human immunoglobulin G content per mL of the reconstituted product

5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains marked precipitates.

5.3 Reconstituted

The reconstituent shall be water for injection.

## **FREEZE-DRIED PEPSIN-TREATED NORMAL HUMAN IMMUNOGLOBULIN**

### **1. Descriptive definition**

“Freeze-dried Pepsin-treated Normal Human Immunoglobulin” is a freeze-dried product containing pepsin-treated fragment of human immunoglobulin G. When reconstituted, it becomes a clear or slightly whitish turbid liquid, and contains almost no visible deposits.

### **2. Production control**

#### 2.1 Source material

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### 2.2 Bulk material

Immunoglobulin G fraction shall be prepared by fractionating source plasma using a method shown to be free from a risk of transmission of hepatitis viruses or other microorganisms and not to cause the deterioration of antibodies. The immunoglobulin G fraction shall be treated with pepsin using a method that could render the concentration of monomeric or higher forms of globulin to not more than 10% of the total proteins. Resulting fragments shall be collected and serve as bulk material.

#### 2.3 Final bulk and final product

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers and freeze-dried. When reconstituted by the method stated on the label, the product should contain pepsin-treated human immunoglobulin G fragment at a concentration of not less than 5 w/v%.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### 3.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### 3.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.4.

#### 3.3 Test for pepsin-treated immunoglobulin G content

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the protein should have the mobility of normal human immunoglobulin G. In addition, when the test for protein nitrogen content given in General Tests is applied, the pepsin-treated immunoglobulin G content per mL calculated from total protein content shall be within 90–110% of the value stated on the label.

#### 3.4 Immunological identification test

When tested by immunoelectrophoresis against antiserum to human serum, the final product shall form a distinct precipitation line of pepsin-treated human immunoglobulin G and no unspecific

lines of precipitation shall be observed.

3.5 Test for freedom from anticomplementary effect

The test given in General Tests shall apply.

3.6 Sterility test

The test given in General Tests shall apply.

3.7 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.8 Pyrogen test

The test given in General Tests shall apply.

3.9 Titration test for measles

The titer shall be no less than 5 units per **100** mg of pepsin human immunoglobulin G, complying with the titration test for measles given in General Tests.

**4. Storage and expiry date**

The expiry date shall be three years.

**5. Other requirements**

5.1 Labeling contents

Pepsin-treated human immunoglobulin G content per mL of the reconstituted product

5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains marked precipitates.

5.3 Reconstituent

The reconstituent shall be water for injection.

# **POLYETHYLENE GLYCOL-TREATED NORMAL HUMAN IMMUNOGLOBULIN**

## **1. Descriptive definition**

“Polyethylene Glycol-treated Normal Human Immunoglobulin” is a clear, colorless or pale yellow liquid product containing polyethylene glycol-treated immunoglobulin G.

## **2. Production control**

### **2.1 Source material**

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

### **2.2 Bulk material**

The source plasma shall be fractionated using a method shown to be free from a risk of transmission of hepatitis viruses or other microorganisms and not to cause the deterioration of antibodies. The immunoglobulin G fraction shall be treated with polyethylene glycol. The material thereby obtained shall serve as bulk material.

### **2.3 Final bulk and final product**

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers. The product should contain human immunoglobulin G at a concentration of not less than 5 w/v%.

## **3. Tests on final product**

Following tests shall apply to each final lot.

### **3.1 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 5.0 to 6.0.

### **3.2 Test for immunoglobulin G content**

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the total protein shall have the mobility of normal human immunoglobulin G. In addition, when the test for protein nitrogen content given in General Tests is applied, the immunoglobulin G content per mL calculated from total protein content shall be within 90–110% of the value stated on the label.

### **3.3 Test for freedom from aggregated immunoglobulin G**

When gel permeation chromatography is conducted using a suitable supporting medium for the fractionation of immunoglobulin G, higher order aggregates more than dimer of immunoglobulin G shall be detected at a concentration of not more than 1.0%.

### **3.4 Immunological identification test**

When tested by immunoelectrophoresis against antiserum to human serum, the final product shall form a distinct precipitation line of normal human immunoglobulin G and no unspecific lines of precipitation shall be observed.

### **3.5 Sterility test**

The test given in General Tests shall apply.

3.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.7 Pyrogen test

The test given in General Tests shall apply.

3.8 Titration test for measles

The titer shall be no less than 5 units per 150 mg of human immunoglobulin G, complying with the titration test for measles given in General Tests.

**4. Storage and expiry date**

The expiry date shall be two years.

**5. Other requirements**

5.1 Labeling contents

Human immunoglobulin G content per mL of the reconstituted product

5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains insoluble matters.

# **FREEZE-DRIED POLYETHYLENE GLYCOL-TREATED NORMAL HUMAN IMMUNOGLOBULIN**

## **1. Descriptive definition**

“Freeze-dried Polyethylene Glycol-treated Normal Human Immunoglobulin” is a freeze-dried product containing polyethylene glycol-treated human immunoglobulin G. When reconstituted, it becomes a pale yellow, clear or slightly turbid liquid, and contains almost no visible deposits.

## **2. Production control**

### **2.1 Source material**

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

### **2.2 Bulk material**

Immunoglobulin G fraction shall be prepared by fractionating source plasma using a method shown to be free from a risk of transmission of hepatitis viruses or other microorganisms and not to cause the deterioration of antibodies. The immunoglobulin G fraction shall be treated with polyethylene glycol, or, following polyethylene glycol treatment, hydroxyethyl starch. The material thereby obtained shall serve as bulk material.

### **2.3 Final bulk and final product**

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers and freeze-dried. When reconstituted by the method stated on the label, the product should contain human immunoglobulin G at a concentration of not less than 5 w/v%.

## **3. Tests on final product**

Following tests shall apply to each final lot.

### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

### **3.3 Test for immunoglobulin G content**

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the total protein, from which added human serum albumin is excluded, shall have the mobility of normal human immunoglobulin G. In addition, when the test for protein nitrogen content given in General Tests is applied, the polyethylene glycol-treated immunoglobulin G content per mL calculated from total protein content shall be within 90–110% of the value stated on the label.

### **3.4 Test for freedom from aggregated immunoglobulin G**

Requirements given in 3.3 of “Polyethylene Glycol-treated Normal Human Immunoglobulin” shall apply.

### **3.5 Immunological identification test**

Requirements given in 3.4 of “Polyethylene Glycol-treated Normal Human Immunoglobulin” shall apply.

3.6 Test for freedom from anti-complementary effect

The test given in General Tests shall apply.

3.7 Sterility test

The test given in General Tests shall apply.

3.8 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.9 Pyrogen test

The test given in General Tests shall apply.

3.10 Titration test for measles

Requirements given in 3.8 of “Polyethylene Glycol-treated Normal Human Immunoglobulin” shall apply.

**4. Storage and expiry date**

The expiry date shall be two years.

**5. Other requirements**

5.1 Labeling contents

Human immunoglobulin G content per mL of the reconstituted product

5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains marked precipitates.

5.3 Reconstituent

The reconstituent shall be water for injection.

## HUMAN ANTI-HBs IMMUNOGLOBULIN

### 1. Descriptive definition

“Human Anti-HBs Immunoglobulin” is a colorless or yellowish-brown clear liquid containing "anti-HBs antibody" in human Immunoglobulin G.

### 2. Production control

#### 2.1 Source material

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply. Healthy, anti-HBs antibody-positive individuals with no laboratory evidence of HBs antigen shall be selected as donors.

#### 2.2 Bulk material

Immunoglobulin G fraction shall be obtained by fractionating source plasma using a method shown not to cause the deterioration of antibodies.

#### 2.3 Final bulk and final product

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material and then distributed into final containers. The final bulk should contain anti-HBs antibody at a concentration of no less than 200 units per mL. If necessary, an appropriate preservative can be added

### 3. Tests on final product

Following tests shall apply to each final lot, except for the test given in 3.2 if no preservative is used

#### 3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

#### 3.2 Test for thimerosal content

The test given in General Tests shall apply if thimerosal is added as a preservative. The thimerosal content shall be no more than 0.012 w/v%.

#### 3.3 Test for immunoglobulin G content

When the electrophoretic test given in General Tests is applied, no less than 90% of the total protein shall have the mobility of normal human immunoglobulin G.

#### 3.4 Identity test

The test given in 3.4 of Normal Human Immunoglobulin shall apply.

#### 3.5 Sterility test

The test given in General Tests shall apply.

#### 3.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.7 Pyrogen test

The test given in General Tests shall apply. The dose of application is 1.0 mL/kg body weight.

#### 3.8 Potency test

The test given in General Tests shall apply. The anti-HBs antibody content shall be no less than

200 units per mL and no less than the value stated on the label.

**4. Storage and expiry date**

The expiry date shall be two years.

**5. Other requirements**

5.1 Labeling contents

- (1) Anti-HBs antibody content per mL
- (2) The product shall not be injected intravenously.
- (3) The product shall not be administered to HBs antigen-positive individuals.
- (4) Name and amount of preservative, if used.

5.2 Information to be provided in package insert and other labeling

When preservative has not been used, this shall be stated.

## **FREEZE-DRIED HUMAN ANTI-HBs IMMUNOGLOBULIN**

### **1. Descriptive definition**

“Freeze-dried Human Anti-HBs Immunoglobulin” is a freeze-dried product containing "anti-HBs antibody" in human immunoglobulin G. When reconstituted, it becomes a colorless, yellowish-brown clear or slightly whitish turbid liquid.

### **2. Production control**

#### **2.1 Source material**

Requirements given in 2.1 of Human Anti-HBs Immunoglobulin shall apply.

#### **2.2 Bulk material**

Requirements given in 2.2 of Human Anti-HBs Immunoglobulin shall apply.

#### **2.3 Final bulk and freeze-drying**

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents and distributed into final containers and freeze-dried. When reconstituted by the method stated on the label, the product should contain anti-HBs antibody at a concentration of no less than 200 units per mL.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

#### **3.3 Test for immunoglobulin G content**

When the electrophoretic test given in General Tests is applied, no less than 90% of the total protein shall have the mobility of normal human immunoglobulin G.

#### **3.4 Identity test**

The tests given in 3.4 of Normal Human Immunoglobulin shall apply.

#### **3.5 Sterility test**

The test given in General Tests shall apply.

#### **3.6 Test for freedom from abnormal toxicity**

The test given in General Tests shall apply.

#### **3.7 Pyrogen test**

The test given in General Tests shall apply. The dose of application is 1.0 mL/kg body weight.

#### **3.8 Potency test**

The test given in 3.8 of Human Anti-HBs Immunoglobulin shall apply.

### **4. Storage and expiry date**

The expiry date shall be five years.

## **5. Other requirements**

### 5.1 Labeling contents

- (1) Anti-HBs antibody content per mL
- (2) The product shall not be injected intravenously.
- (3) The product shall not be administered to HBs antigen-positive individuals.

### 5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains marked precipitates.

### 5.3 Reconstituent

The reconstituent shall be sterile water for injection.

## **POLYETHYLENE GLYCOL-TREATED HUMAN ANTI-HBs IMMUNOGLOBULIN**

### **1. Descriptive definition**

“Polyethylene Glycol-treated Human Anti-HBs Immunoglobulin” is a colorless or pale yellow clear liquid containing “Polyethylene Glycol-treated anti-HBs Antibody” in human Immunoglobulin G.

### **2. Production control**

#### **2.1 Source material**

Requirements given in 2.1 of Human Anti-HBs Immunoglobulin shall apply.

#### **2.2 Bulk material**

Immunoglobulin G fraction shall be prepared by fractionating source plasma using a method capable of eliminating pathogenic microbes, including hepatitis viruses, to the greatest extent possible and shown not to cause the deterioration of antibodies. This fraction shall be treated with polyethylene glycol and the resulting material shall serve as bulk material.

#### **2.3 Final bulk and final product**

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents and distributed into final containers. The product should contain anti-HBs antibody at a concentration of no less than 200 units per mL.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### **3.1 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 5.0 to 6.0.

#### **3.2 Test for immunoglobulin G content**

When the electrophoretic test given in General Tests is applied, no less than 90% of the total protein shall have the mobility of normal human immunoglobulin G.

#### **3.3 Test for freedom from aggregated immunoglobulin G.**

The test given in 3.3 of “Polyethylene Glycol-treated Normal Human Immunoglobulin” shall apply.

#### **3.4 Identity test**

The test given in 3.4 of “Polyethylene Glycol-treated Normal Human Immunoglobulin” shall apply.

#### **3.5 Sterility test**

The test given in General Tests shall apply.

#### **3.6 Test for freedom from abnormal toxicity**

The test given in General Tests shall apply.

#### **3.7 Pyrogen test**

The test given in General Tests shall apply.

#### **3.8 Potency test**

The test given in 3.8 of “Human anti-HBs Immunoglobulin” shall apply.

**4. Storage and expiry date**

The expiry date shall be three years.

**5. Other requirements**

5.1 Labeling contents

(1) Anti-HBs antibody content per mL

(2) The product shall not be administered to HBs antigen-positive individuals.

5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains insoluble matters.

# **FREEZE-DRIED POLYETHYLENE GLYCOL-TREATED HUMAN ANTI-HBs IMMUNOGLOBULIN**

## **1. Descriptive definition**

“Freeze-dried Polyethylene Glycol-treated Human Anti-HBs Immunoglobulin” is a freeze-dried product containing “Polyethylene Glycol-treated anti-HBs Antibody” in human Immunoglobulin G. When reconstituted, it becomes a pale yellow, clear or slightly turbid liquid, and contains almost no visible deposits.

## **2. Production control**

### **2.1 Source material**

Requirements given in 2.1 of “Human Anti-HBs Immunoglobulin” shall apply.

### **2.2 Bulk material**

Immunoglobulin G fraction shall be obtained by fractionating source plasma using a method capable of eliminating pathogenic microbes, including hepatitis viruses, to the greatest extent possible and has been shown not to cause the deterioration of antibodies. This fraction shall be treated with polyethylene glycol and the resulting material shall serve as bulk material.

### **2.3 Final bulk and final product**

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents and distributed into final containers and freeze-dried. When reconstituted by the method stated on the label, the product should contain anti-HBs antibody at a concentration of no less than 200 units per mL.

## **3. Tests on final product**

Following tests shall apply to each final lot.

### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

### **3.3 Test for immunoglobulin G content**

When the electrophoretic test given in General Tests is applied, no less than 90% of the total protein, from which added human serum albumin is excluded, shall have the mobility of normal human immunoglobulin G.

### **3.4 Test for freedom from aggregated immunoglobulin G.**

The test given in 3.3 of “Polyethylene Glycol-treated Normal Human Immunoglobulin” shall apply.

### **3.5 Identity test**

The test given in 3.4 of “Polyethylene Glycol-treated Normal Human Immunoglobulin” shall apply.

### **3.6 Test for freedom from anti-complementary effect**

The test given in General Tests shall apply.

3.7 Sterility test

The test given in General Tests shall apply.

3.8 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.9 Pyrogen test

The test given in General Tests shall apply.

3.10 Potency test

The test given in 3.8 of Human anti-HBs Immunoglobulin shall apply.

**4. Storage and expiry date**

The expiry date shall be three years.

**5. Other requirements**

5.1 Labeling contents

(1) Anti-HBs antibody content per mL of the reconstituted product

(2) The product shall not be administered to HBs antigen-positive individuals.

5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains marked precipitates.

5.3 Reconstituent

The reconstituent shall be sterile water for injection.

## HUMAN ANTI-D(Rho) IMMUNOGLOBULIN

### 1. Descriptive definition

“Human Anti-D(Rho) Immunoglobulin” is a colorless or yellowish-brown clear liquid product containing "Anti-D(Rho) antibody" in human immunoglobulin G.

### 2. Production control

#### 2.1 Source material

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply. Individuals having antibodies to D(Rho) factor shall be selected as donors.

#### 2.2 Fractionation

Immunoglobulin G fraction shall be obtained by fractionating source plasma using a method shown not to cause the deterioration of antibodies.

#### 2.3 Final bulk and final product

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents and distributed into final containers. The anti-D(Rho) titer shall not be less than 2,000. If necessary, a suitable preservative can be added

### 3. Tests on final product

Following tests shall apply to each final lot, except for the test given in 3.2 if no preservative is used

#### 3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.6.

#### 3.2 Test for thimerosal content

If thimerosal has been used as a preservative, the test given in General Tests shall apply and the thimerosal content shall be no more than 0.012 w/v%.

#### 3.3 Test for immunoglobulin G content

When the electrophoretic test given in General Tests is applied, no less than 90% of the total protein shall have the mobility of normal human immunoglobulin G.

#### 3.4 Identity test

The test given in 3.4 of Normal Human Immunoglobulin shall apply.

#### 3.5 Sterility test

The test given in General Tests shall apply.

#### 3.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.7 Pyrogen test

The test given in General Tests shall apply. The dose of application is 1.0 mL/kg body weight.

#### 3.8 Potency test

The test given in General Tests shall apply. Anti-D(Rho) titer shall be no less than 2,000 and no less than the value stated on the label.

**4. Storage and expiry date**

The expiry date shall be six months.

**5. Other requirements**

5.1 Labeling contents

- (1) Anti-D(Rho) titer
- (2) The product shall not be administered to newborn infants.
- (3) The product shall be given only to D(Rho)-negative females who have not yet been sensitized to D(Rho) factors, within 72 hours of delivery
- (4) The product shall not be injected intravenously.
- (5) Name and amount of preservative, if used.

5.2 Information to be provided in package insert and other labeling

When preservative has not been used, this shall be stated.

## **FREEZE-DRIED HUMAN ANTI-D(Rho) IMMUNOGLOBULIN**

### **1. Descriptive definition**

“Freeze-dried Human Anti-D(Rho) Immunoglobulin” is a freeze-dried product containing "anti-D(Rho) antibody" in human immunoglobulin G. When reconstituted, it becomes slightly whitish turbid liquid.

### **2. Production control**

#### **2.1 Source material**

Requirements given in 2.1 of “Human Anti-D(Rho) Immunoglobulin” shall apply.

#### **2.2 Fractionation**

Immunoglobulin G fraction shall be obtained by fractionating source plasma using a method shown not to cause the deterioration of antibodies.

#### **2.3 Final bulk and final product**

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents and distributed into final containers and freeze-dried. When the product is reconstituted by the method stated on the label, the anti-D(Rho) titer shall be no less than 1,000.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.6.

#### **3.3 Test for immunoglobulin G content**

When the electrophoretic test given in General Tests is applied, no less than 90% of the total protein shall have the mobility of normal human immunoglobulin G.

#### **3.4 Identity test**

The test given in 3.4 of “Normal Human Immunoglobulin” shall apply.

#### **3.5 Sterility test**

The test given in General Tests shall apply.

#### **3.6 Test for freedom from abnormal toxicity**

The test given in General Tests shall apply.

#### **3.7 Pyrogen test**

The test given in General Tests shall apply. The dose of application is 1.0 mL/kg body weight.

#### **3.8 Potency test**

The test given in 3.8 of “Human Anti-D(Rho) Immunoglobulin” shall apply. Anti-D(Rho) titer of the appropriately reconstituted product shall be no less than 1,000 and no less than the value stated on the label.

**4. Storage and expiry date**

The expiry date shall be three years.

**5. Other requirements**

5.1 Labeling contents

- (1) Anti-D(Rho) titer of the reconstituted product
- (2) The product shall not be administered to newborn infants.
- (3) The product shall be given only to D(Rho)-negative females who have not yet been sensitized to D(Rho) factor, within 72 hours of delivery;
- (4) The product shall not be injected intravenously.

5.2 Reconstituent

The reconstituent shall be sterile water for injection.

## HUMAN ANTI-TETANUS IMMUNOGLOBULIN

### 1. Descriptive definition

“Human Anti-tetanus Immunoglobulin” is a colorless or pale-yellow clear liquid product containing "tetanus antitoxin" in human immunoglobulin G.

### 2. Production control

#### 2.1 Source material

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply. Healthy individuals who have recently been immunized with tetanus toxoid shall be selected as donors.

#### 2.2 Bulk material

The fraction containing immunoglobulin G shall be prepared by fractionating source plasma using a suitable method shown not to cause the deterioration of antibodies, and the material thereby obtained shall serve as bulk material.

#### 2.3 Final bulk and final product

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers. The product should contain tetanus antitoxin at a concentration of no less than 125 U/mL.

### 3. Tests on final product

Following tests shall apply to each final lot.

#### 3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

#### 3.2 Test for immunoglobulin G content

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the total protein shall have the mobility of normal human immunoglobulin G.

#### 3.3 Immunological identification test

Requirements given in 3.4 of “Normal human immunoglobulin” shall apply.

#### 3.4 Sterility test

The test given in General Tests shall apply.

#### 3.5 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.6 Pyrogen test

The test given in General Tests shall apply. The dose of application is 1.0 mL/kg body weight.

#### 3.7 Potency test

When the potency test in General Tests is applied, the titer shall be no less than 125 units per mL and the value stated on the label. Standard Anti-tetanus Human Immunoglobulin shall be used as

test standard.

**4. Storage and expiry date**

The expiry date shall be two years.

**5. Other requirements**

5.1 Labeling contents

- (1) Tetanus toxoid content per mL
- (2) The product shall not be injected intravenously.

## **FREEZE-DRIED HUMAN ANTI-TETANUS IMMUNOGLOBULIN**

### **1. Descriptive definition**

“Freeze-dried Human Anti-tetanus Immunoglobulin” is a freeze-dried product containing "tetanus antitoxin" in human immunoglobulin G. When reconstituted, it becomes slightly turbid liquid.

### **2. Production control**

#### **2.1 Source material**

Requirements given in 2.1 of “Human Anti-tetanus Immunoglobulin” shall apply.

#### **2.2 Bulk material**

The fraction containing immunoglobulin G shall be prepared by fractionating source plasma using a suitable method shown not to cause the deterioration of antibodies, and the material thereby obtained shall serve as bulk material.

#### **2.3 Final bulk and final product**

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers and freeze-dried. When reconstituted by the method stated on the label, the product should contain tetanus antitoxin at a concentration of no less than 50 units per mL.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

#### **3.3 Test for immunoglobulin G content**

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the total protein should have the mobility of normal human immunoglobulin G.

#### **3.4 Immunological identification test**

Requirements given in 3.4 of Normal Human Immunoglobulin shall apply.

#### **3.5 Sterility test**

The test given in General Tests shall apply.

#### **3.6 Test for freedom from abnormal toxicity**

The test given in General Tests shall apply.

#### **3.7 Pyrogen test**

The test given in General Tests shall apply. The dose of application is 1.0 mL/kg body weight.

#### **3.8 Potency test**

When the potency test in General Tests is applied, the titer shall be no less than 50 units per mL and the value stated on the label. Standard Anti-tetanus Human Immunoglobulin shall be used as test standard.

**4. Storage and expiry date**

The expiry date shall be five years.

**5. Other requirements**

5.1 Labeling contents

- (1) Tetanus toxoid content per mL
- (2) The product shall not be injected intravenously.

5.2 Reconstituent

The reconstituent shall be water for injection.

# **POLYETHYLENE GLYCOL-TREATED HUMAN ANTI-TETANUS IMMUNOGLOBULIN**

## **1. Descriptive definition**

“Polyethylene Glycol-treated Human Anti-tetanus Immunoglobulin” is a colorless or pale-yellow clear liquid product containing "tetanus antitoxin" in polyethylene glycol-treated human immunoglobulin G.

## **2. Production control**

### 2.1 Source material

Requirements given in 2.1 of “Human Anti-tetanus Immunoglobulin” shall apply.

### 2.2 Bulk material

Immunoglobulin G fraction shall be prepared by fractionating source plasma using a method shown to be free from a risk of transmission of hepatitis viruses or other microorganisms and not to cause the deterioration of antibodies. The immunoglobulin G fraction shall be treated with polyethylene glycol. The material thereby obtained shall serve as bulk material.

### 2.3 Final bulk and final product

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers. The product should contain tetanus antitoxin at a concentration of no less than 75 units per mL.

## **3. Tests on final product**

Following tests shall apply to each final lot.

### 3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 5.0 to 6.0.

### 3.2 Test for immunoglobulin G content

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the total protein shall have the mobility of normal human immunoglobulin G.

### 3.3 Test for freedom from aggregated immunoglobulin G

Requirements given in 3.3 of “Polyethylene Glycol-treated Normal Human Immunoglobulin” shall apply.

### 3.4 Immunological identification test

Requirements given in 3.4 of “Polyethylene Glycol-treated Normal Human Immunoglobulin” shall apply.

### 3.5 Sterility test

The test given in General Tests shall apply.

### 3.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.7 Pyrogen test

The test given in General Tests shall apply. The dose of application is 1.0 mL/kg body weight.

### 3.8 Potency test

When the potency test in General Tests is applied, the titer shall be no less than 75 units per mL and the value stated on the label. Standard Anti-tetanus Immunoglobulin shall be used as test standard.

## 4. Storage and expiry date

The expiry date shall be two years.

## 5. Other requirements

### 5.1 Labeling contents

Tetanus toxoid content per mL

### 5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains insoluble matters.

## **FREEZE-DRIED POLYETHYLENE GLYCOL-TREATED HUMAN ANTI-TETANUS IMMUNOGLOBULIN**

### **1. Descriptive definition**

“Freeze-dried Polyethylene Glycol-treated Human Anti-tetanus Immunoglobulin” is a freeze-dried product containing polyethylene glycol-treated human anti-tetanus immunoglobulin. When reconstituted, it becomes a pale-yellow clear or slightly turbid liquid, and contains almost no visible deposits.

### **2. Production control**

#### **2.1 Source material**

Requirements given in 2.1 of “Human Anti-tetanus Immunoglobulin” shall apply.

#### **2.2 Bulk material**

Immunoglobulin G fraction shall be prepared by fractionating source plasma using a method shown to be free from a risk of transmission of hepatitis viruses or other microorganisms and not to cause the deterioration of antibodies. The immunoglobulin G fraction shall be treated with polyethylene glycol. The material thereby obtained shall serve as bulk material.

#### **2.3 Final bulk and final product**

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to bulk material. The final bulk shall then be distributed into final containers and freeze-dried. When reconstituted by the method stated on the label, the product should contain tetanus antitoxin at a concentration of no less than 75 units per mL.

### **3. Tests on final product**

Following tests shall apply to each final lot.

#### **3.1 Test for moisture content**

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### **3.2 Test for pH**

When the test given in General Tests is applied, the pH shall be within the range of 6.4 to 7.2.

#### **3.3 Test for immunoglobulin G content**

When the cellulose acetate membrane electrophoretic analysis given in General Tests is applied, no less than 90% of the total protein, from which added human serum albumin is excluded, shall have the mobility of normal human immunoglobulin G.

#### **3.4 Test for freedom from aggregated immunoglobulin G**

Requirements given in 3.3 of “Polyethylene Glycol-treated Normal Human Immunoglobulin” shall apply.

#### **3.5 Immunological identification test**

Requirements given in 3.4 of “Polyethylene Glycol-treated Normal Human Immunoglobulin” shall apply.

3.6 Test for freedom from anti-complementary effect

The test given in General Tests shall apply.

3.7 Sterility test

The test given in General Tests shall apply.

3.8 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.9 Pyrogen test

The test given in General Tests shall apply.

3.10 Potency test

When the potency test in General Tests is applied, the titer shall be no less than 75 units per mL and the value stated on the label. Standard Anti-tetanus Human Immunoglobulin shall be used as test standard.

**4. Storage and expiry date**

The expiry date shall be two years.

**5. Other requirements**

5.1 Labeling contents

Tetanus toxoid content per mL

5.2 Information to be provided in package insert and other labeling

The product shall not be used if the reconstituted solution contains marked precipitates.

5.3 Reconstituent

The reconstituent shall be water for injection.

## **FREEZE-DRIED HUMAN ANTITHROMBIN III CONCENTRATE**

### **1. Descriptive definition**

“Freeze-dried Human Antithrombin III Concentrate” is a preparation of a fraction obtained from human plasma that contains antithrombin III. When reconstituted, it becomes a colorless or pale yellow, clear or slightly turbid liquid.

### **2. Production control**

#### 2.1 Source material

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### 2.2 Bulk material

Source plasma is fractionated by a suitable method shown not to denature antithrombin III. The collected antithrombin III fraction serves as bulk material.

#### 2.3 Final bulk and final product

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers and freeze-dried.

### **3. Tests on the final product**

Following tests shall apply to each final lot.

#### 3.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### 3.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.5 to 8.0.

#### 3.3 Test for protein content

When the test given in General Tests is applied, the protein content shall be no more than 10 mg per 25 units.

#### 3.4 Immunological identification test

When tested by immunoelectrophoresis against antiserum to human antithrombin III, the final product shall form distinct precipitation line of normal human immunoglobulin G and no unspecific lines of precipitation shall be observed.

#### 3.5 Sterility test

The test given in General Tests shall apply.

#### 3.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

#### 3.7 Pyrogen test

The test given in General Tests shall apply.

#### 3.8 Potency test

Test sample solution and Concentrated Antithrombin III International Standard or its Reference solution shall be prepared by accurate two-fold logarithmic dilutions with a suitable buffer containing appropriate amounts of sodium heparin and human serum albumin. To the accurately measured test sample solution and Reference solution, a given amount of thrombin shall be added, mixed, and incubated at  $37.0 \pm 0.5^{\circ}\text{C}$  for a certain period to permit reaction, and the residual thrombin activity shall be determined using a suitable chromogenic substance. The amount of thrombin inactivated by antithrombin III shall be proportional to the difference in the thrombin activity in the reaction of each sample and the Reference solution. The estimated potency shall be no less than 10 units per mL and no less than the potency stated on the label.

#### **4. Storage and expiry date**

The expiry date shall be two years.

#### **5. Other requirements**

##### **5.1 Labeling contents**

Amount of antithrombin III per mL in reconstituted solution.

##### **5.2 Information to be provided in package insert and other labeling**

The product shall not be used if the reconstituted solution contains marked precipitates.

##### **5.3 Reconstituent**

The reconstituent shall be water for injection.

## **FREEZE-DRIED HUMAN ACTIVATED PROTEIN C CONCENTRATE**

### **1. Descriptive definition**

“Freeze-dried Human Activated Protein C Concentrate” is a freeze-dried product containing human activated protein C. When reconstituted, it becomes a colorless or pale yellow, clear liquid.

### **2. Production control**

#### 2.1 Source material

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### 2.2 Bulk material

Source plasma shall be fractionated by a suitable method that does not cause the denaturation of protein C. The fraction containing protein C shall be activated with a suitable activating reagent, and then the activating reagent shall be removed. The resulting fraction shall serve as bulk material.

#### 2.3 Final bulk and final product

Final bulk shall be prepared by adding appropriate stabilizing and isotonicizing agents to the bulk material. The final bulk shall then be distributed into final containers and freeze-dried.

### **3. Tests on the final product**

Following tests shall apply to each final lot.

#### 3.1 Test for moisture content

Moisture content shall be no higher than 3.0% when tested according to the test given in General Tests.

#### 3.2 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.7 to 7.3.

#### 3.3 Identification test

Test sample shall be diluted with a suitable buffer containing bovine serum albumin. To two micro-well plates coated with either a monoclonal antibody against protein C or polyclonal antibody against human protein C, 100  $\mu$ L of the dilution buffer, followed by 20  $\mu$ L of either the diluted sample or the dilution buffer shall be added and incubated. The contents of the wells shall be aspirated and the wells shall be washed. Then, 150  $\mu$ L of enzyme-labeled anti-human protein C antibody solution shall be added to the wells and incubated. The contents of the wells shall be aspirated and the wells washed. To the wells, 150  $\mu$ L of the chromogenic substrate solution shall be added and the plates shall be left for 30 minutes at room temperature, and protected from light. The reaction shall be terminated by adding 50  $\mu$ L of stop solution, and the resulting color shall be observed visually. The diluted test sample should not show any color development on the plate coated with monoclonal antibody against protein C, but should show color development on the plate coated with polyclonal antibody against human protein C. The control should not show color development on either plate.

#### 3.4 Test for freedom from active coagulation factor

A 0.4-mL portion of the test sample shall be added to 20  $\mu$ L of 0.6 mol/L calcium chloride solution and 0.4 mL of 0.25 w/v% fibrinogen solution in a test tube and mixed. The tube shall be placed in a water bath at 37°C, at which time the macroscopic fibrinogen coagulation test shall be started upon the placement of the tube in the water bath. The control reaction mixture shall be prepared by using physiological saline instead of the test sample. Neither the test sample nor the control should show coagulation in less than 24 hours.

### 3.5 Sterility test

The test given in General Tests shall apply.

### 3.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

### 3.7 Pyrogen test

The test given in General Tests shall apply.

### 3.8 Potency test

Test sample and standard for activated protein C potency test shall be diluted with a suitable buffer containing bovine serum albumin. To these dilutions, add normal human plasma prepared from more than 10 donors, followed by activated partial thromboplastin solution. The mixture shall be immediately incubated at 36.5–37.5°C for a defined period, and 0.025 mol/L calcium chloride solution shall be added to measure the clotting time. For measurement, the diluted test sample or diluted standard, normal human plasma, activated partial thromboplastin solution and 0.025 mol/L calcium chloride solution shall be mixed in exactly a same amount in the range of 75 to 100  $\mu$ L. The control clotting time shall be measured in the similar manner by using the buffer instead of diluted samples. The potency of the test sample shall be calculated based on the clotting time for the diluted sample and diluted standard. The estimated potency shall be in the range of 80 to 140% of the potency stated on the label.

## 4. Storage and expiry date

The expiry date shall be three years.

## 5. Other requirements

### 5.1 Labeling contents

Amount of activated protein C per mL of the reconstituted solution

### 5.2 Reconstituent

The reconstituent shall be water for injection.

## HUMAN HAPTOGLOBIN

### 1. Descriptive definition

“Human Haptoglobin” is an amber-colored clear liquid that contains human serum haptoglobin.

### 2. Production control

#### 2.1 Source material

Requirements given in 1-4 of General Notices and 2-2-(6),(7) in General Rules for Blood Products shall apply.

#### 2.2 Bulk material

Source plasma shall be fractionated by a suitable method which causes a minimal decrease in hemoglobin binding activity and is capable of eliminating hepatitis viruses and other microorganisms to the greatest extent possible. The fraction containing haptoglobin shall be collected, mixed with a suitable stabilizer, if necessary, and heat-treated at  $60.0 \pm 0.5^{\circ}\text{C}$  for more than 10 hours. The resulting material shall serve as bulk material.

#### 2.3 Final bulk and final product

Final bulk shall be prepared by adding a suitable solution to the bulk material. The final bulk shall then be distributed into final containers.

### 3. Tests on final product

Following tests shall apply to each final lot.

#### 3.1 Test for pH

When the test given in General Tests is applied, the pH shall be within the range of 6.0 to 7.5.

#### 3.2 Test for protein content

When the test given in General Tests is applied, the protein content shall be no more than 50 mg/mL

#### 3.3 Identification test

When tested by double immunodiffusion method utilizing antisera specific to human haptoglobin, the final product shall form a distinct precipitation line of normal human haptoglobin.

#### 3.4 Test for hemoglobin content

Hemoglobin content shall be measured by the van Kampen reaction. A 0.025-w/v% cyanmethemoglobin standard solution (hereafter referred to as the “standard solution” in this monograph) shall be prepared by diluting the cyanmethemoglobin standard solution with water. To accurately measure 1-mL portion of the four-fold dilution of the test sample and standard, 4mL of water shall be added and the mixture shall be kept standing at room temperature for 5 minutes. The absorbance shall then be measured at 540 nm with a spectrophotometer. The  $A_{540}$  of the four-fold diluted sample solution shall be no more than the  $A_{540}$  of the standard solution.

#### 3.5 Sterility test

The test given in General Tests shall apply.

3.6 Test for freedom from abnormal toxicity

The test given in General Tests shall apply.

3.7 Pyrogen test

The test given in General Tests shall apply.

3.8 Potency

Potency of haptoglobin shall be determined by measuring its activity in binding to hemoglobin. The test sample, either undiluted or diluted in a suitable concentration, shall be used in the determination. To prepare the test sample solution, the test sample is taken in an amount of 0.36–0.45 mL on a 0.01 mL basis; 0.1 mL of an 80 mg/mL hemoglobin solution or 0.1 mL of hemoglobin solution diluted at the same dilution ratio as the test sample is added; and then a sufficient amount of physiological saline is added to make 0.55 mL. The test sample solution shall be subjected to electrophoretic analysis using a suitable supporting medium, for example, cellulose acetate membrane or polyacrylamide gel, followed by staining with a suitable dye, for example, o-dianicidine or 2,7-diaminofluoren dihydrochloride. The hemoglobin binding activity of haptoglobin shall be calculated by using a minimum volume of the test sample solution that does not contain free hemoglobin. The potency shall be calculated by using the least volume of preparation in the sample for which no free hemoglobin is detected. One unit of haptoglobin is equal to the amount of haptoglobin which binds to 1mg of hemoglobin. The hemoglobin binding activity per mL of the sample preparation shall be in the range of 90–110% of the activity stated on the label.

**4. Storage and expiry date**

The expiry date shall be two years.

**5. Labeling contents**

Amount of haptoglobin per mL

## **General Tests**

### **A. Test Procedures**

#### **Test for Aluminum Content**

Aluminum content is determined by measuring the color developed by the reaction with stilbazo. The test is conducted after the insoluble salt of aluminum contained in the test sample is solubilized.

##### **Test Procedure**

Shake the test sample to make a homogeneous suspension and transfer 1 mL of the suspension accurately. To this suspension, add 0.2 mL of 1 mol/L sodium hydroxide solution or 1 mol/L nitric acid solution and dissolve insoluble substances. Dilute the sample accurately with water to make a test dilution at a concentration in the range between the highest and lowest concentrations of standard dilutions.

Dilute the 0.1 w/v% aluminum standard solution with water to make not fewer than three different concentrations of the standard dilutions. Transfer a 1-mL portion of each of the test dilutions and the standard dilutions accurately. Add 2.5 mL of water, 1 mL of 1 mol/L acetic acid buffer solution and 0.5 mL of the stilbazo test solution accurately. Allow the mixtures to stand at room temperature for 20 minutes and then determine the absorbance at 510 nm with a spectrophotometer immediately.

Determine the aluminum content of the test dilution from the calibration curve obtained with the standard solutions, and calculate the aluminum content in each mL of the test sample.

Correct the result by measuring the absorbance of water treated in the same manner as above.

## **Test for Freedom from Abnormal Toxicity (General Safety Tests)**

The test is conducted by the following procedure, unless otherwise specified.

### **Animals**

Guinea pigs weighing 300–400 g which show no signs of disease and a normal increase in body weight during a quarantine period of at least 5 days are used.

### **Test dose**

Five milliliters per animal is used, unless otherwise specified.

### **Test procedure**

Animals in the number required for statistical analysis are used for the test with each test sample. The test sample is given by intraperitoneal injection once, and the animals are observed for a period of not less than 7 days. The control group consisting of the same number of animals is prepared and treated with physiological saline in a same manner. The saline control group may be replaced by a pooled group consisting of a statistically sufficient number of animals injected with the same product.

### **Criterion for judgment**

None of the animals shall show any abnormal signs, including body weight loss, during the observation period. No statistically significant ( $p=0.01$ ) difference in weight loss is observed between the treated animals and the control group on any observation day. These criteria are also applied if the product-injected pooled animals described above are used as the control. When a statistically significant difference in weight loss is observed, the test is repeated up to twice. If a statistically significant weight loss is still observed in the second test, histopathological examination is conducted and the results included in the judgment. A product which has caused a loss of weight greater than that by the control treatment due to a hematologic property specific to the product is not required to be subjected to histopathological evaluation.

## Test for Chloride Content

Chloride ion in the test sample is allowed to react with silver ion generated by electrolysis, and the chloride content is determined by “1. Coulometric titration” or “2. Volumetric titration method”, by measuring the amount of electricity required for generation. The criterion for judgment shall be given in the individual monographs.

### 1. Coulometric titration method

#### Test procedure

Prepare an appropriate concentration of chlorine standard solution with sodium chloride. Transfer a fixed quantity of the chlorine standard solution into a suitable electrolyte solution and use this solution for calibration of coulometric titrator comprising a silver electrolysis electrode and a silver ion sensor electrode.

Then, transfer an appropriate amount of test sample into a suitable electrolyte. Determine the chlorine content using the calibrated coulometric titrator by calculating the quantity of electricity consumed for silver ion generation corresponding to the chlorine ion in the test sample.

### 2. Volumetric titration method

#### Test procedure

Transfer an appropriate amount of test sample accurately and add nitric acid·acetic acid test solution. Titrate the test solution with 0.01 mol/L silver nitrate solution according to the method for titration of endpoint detection given in the JP.

0.01 mol/L silver nitrate solution 1mL = 0.35453 mg Cl

## **Bacterial Endotoxins Test**

Bacterial Endotoxins Test is a test to detect or quantify bacterial endotoxins of gram-negative bacterial origin based on the clotting reaction of blood corpuscle lysate of the horse shoe crab (*Limulus polyphemus* or *Tachypleus tridentatus* etc.). The bacterial Endotoxins Test test shall be done by the method given below, unless otherwise specified. The criterion for judgment shall be given in the individual monographs.

### **Test procedure**

The method of the Bacterial Endotoxins Test of the JP shall apply.

Japanese Reference Standard Endotoxin or an appropriate reference endotoxin shall be used as the standard endotoxin. For the detecting endotoxin, an endotoxin-specific lysate reagent, appropriate dilutions of the standard endotoxin, and samples for accurate measurement shall be used. The interfering effect of the test sample shall be appropriately evaluated for individual test samples. Pre-treatment to eliminate the interfering effect of a sample can be done, if necessary.

### **Criterion for judgment**

Endotoxin content of a test sample shall be calculated relative to the potency value of the standard endotoxin by using the parallel line assay method to obtain endotoxin units per mL (EU/mL). The calculated result of the test sample shall not exceed the limit value specified in the monograph.

## **Test for Potassium Content**

Potassium content is determined with a flame spectrophotometer or an atomic absorption spectrophotometer. The criterion for judgment shall be given in the individual monographs.

### **Test procedure**

Transfer a suitable quantity of test sample and dilute it with water or appropriate diluents, if necessary. Measure the brightness of the test material at 766 nm in a flame spectrophotometer or the absorbance at 766 nm in an atomic absorption spectrophotometer.

Dilute potassium standard dilution accurately to make not fewer than three different concentrations of the standard dilutions. Dilute the sample accurately to make a test dilution at a concentration in the range between the highest and lowest concentrations of the standard dilutions.

Determine the brightness or absorbance of the test solution and standard dilutions. Calculate the potassium content in a test sample using the calibration curve obtained from the standard dilutions.

## Test for Moisture Content

The moisture content is determined by “1. Loss on drying test” or “2. Water determination test” unless otherwise specified.

Assessment can be done by one or both methods; however, when doubt exists about the result, the result for 1. Loss on drying test shall be considered final.

### 1. Loss on drying test

The loss on drying method is a method to measure the sample weight loss as a quantity of water by drying under heating and reduced pressure.

#### Test procedure

Dry a weighing bottle with a stopper provided with a capillary of 0.20–0.25 mm inside diameter for 30 minutes in the same manner as the test sample and measure the weight accurately.

Unless otherwise specified, crush the sample, transfer it to weighing bottle and weigh accurately to obtain the net weight of the test sample at a relative humidity of not higher than 45%. Dry the loaded bottle over phosphorus pentoxide (P<sub>2</sub>O<sub>5</sub>) or silica gel at a pressure of not more than 0.6 kPa and at 58–62°C for 3 hours. Place in a desiccator containing P<sub>2</sub>O<sub>5</sub> or silica gel, allow to cool to room temperature and weigh accurately.

The moisture content is calculated by the following formula:

$$\text{Moisture content (\%)} = \frac{\text{weight loss of the test sample by drying (mg)}}{\text{weight of the test sample before drying (mg)}} \times 100$$

### 2. Water determination

The water determination test is a method to determine the water content in a sample material by the Karl Fischer method.

#### Test procedure

Transfer an appropriate amount of test sample accurately and determine the water content in the sample materials according to the method for water determination specified in the JP.

The moisture content is calculated by the following formula:

$$\text{Moisture content (\%)} = \frac{\text{Water amount of the sample (mg)}}{\text{Weight of the sample collected (mg)}} \times 100$$

## Test for Citric Acid Content

Citric acid content is determined by measuring the amount of sodium hydroxide necessary to neutralize the citric acid in the test sample. The criterion for judgment shall be given in the individual monographs.

### Test procedure

Transfer a suitable quantity of the test sample accurately, add water if necessary, and titrate with a 0.1 mol/L sodium hydroxide solution using phenolphthalein test solution as an indicator. The potentiometric titration method in the JP can be also used for titration without the use of an indicator.

The citric acid content in test sample is calculated by the following formula:

$$\text{Citric acid monohydrate content (w/v\%)} = \frac{A \times f \times 0.7005}{B}$$

where A: the volume of 0.1 mol/L sodium hydroxide solution (mL)

f: the factor of 0.1 mol/L sodium hydroxide solution

B: the volume of the test sample (mL).

The citric acid content in the test sample containing sodium dihydrogenphosphate dihydrate is calculated by the following formula:

$$\text{Citric acid monohydrate content (w/v\%)} = \frac{A \times f \times 0.7005}{B} - C \times 0.4490$$

where A: the volume of a 0.1 mol/L sodium hydroxide solution (mL)

f: the factor of 0.1 mol/L sodium hydroxide solution

B: the volume of the test sample (mL)

C: sodium dihydrogenphosphate dihydrate content (w/v%) as determined by the test for sodium dihydrogenphosphate content

## Test for Sodium Citrate Content

Sodium citrate content is determined from the difference between the total citric acid content and the free citric acid content of a test sample by using “1. Weight method” or “2. Liquid chromatography method”. The criterion for judgment shall be given in the individual monographs.

### 1. Weight method

#### Test procedure

Transfer an amount of the test sample expected to contain approximately 150 mg of total citric acid accurately and make-up to approximately 30 mL by adding water. Add 2.0 g of potassium bromide, dissolve completely, and then add 5.0 mL of sulfuric acid. Keep the mixture standing for 5 minutes, gradually add approximately 20 mL of a 5 w/v% potassium permanganate solution and then shake. Keep the mixture standing for 5 minutes and cool to approximately 15°C. To the mixture, add an amount of the ferrous sulfate test solution sufficient to dissolve completely the manganese dioxide precipitate produced during the above treatment, shake, then add 20.0 g of sodium sulfate anhydride and shake vigorously for 2–3 minutes. Collect the resulting crystalline precipitate of pentabromoacetone into a flask by suction with a glass filter covered with asbestos (for Gooch crucible) to an approximately 1mm thickness. Wash the inside of the flask 2–3 times with approximately 25-mL water each time. Filter the washings by suction in the same manner as above. Perform all the above treatments at a temperature of approximately 15°C.

Dry the precipitate on the filter in a sulfuric acid desiccator for approximately 24 hours. Weigh the filter precisely and take the value as A. Then wash the precipitate on the filter off completely about three times with alternating use of ether and ethanol, dry at approximately 100°C for 10 minutes, and cool in a sulfuric acid desiccator. Weigh the filter precisely and take the value as B. The total citric acid content is calculated by the following formula:

$$\text{Total citric acid monohydrate (w/v\%)} = (A - B) \times \frac{0.464}{C} \times 100$$

where C: the volume of test sample (mL)

The sodium citrate content in the test sample is calculated by the following formula. Correction is not made for test sample without free citric acid.

$$\text{Sodium citrate (dehydrate) content (w/v\%)} = [\text{the total citric acid (monohydrate)} - D] \times 1.3995$$

where D: free citric acid (monohydrate) content (w/v%) obtained by the citric acid quantitative method.

### 2. Liquid chromatography

Transfer an appropriate amount of test sample accurately and, if necessary, remove protein in the test sample by an appropriate method. To this solution, add a fixed amount of the internal standard solution and water, if necessary, to make the test sample solution. Transfer an appropriate amount of citric acid accurately and prepare the standard solution in the same manner as the test sample solution. Transfer a fixed amount of test sample solution and standard solution and perform the test under the following conditions according to the liquid chromatography method specified in the JP. Determine the ratio of the peak area of citric acid to that of the internal standard in the test

sample ( $Q_T$ ) and in the standard solution ( $Q_S$ ). Calculate the total amount of citrate by the following formula:

$$\text{Citric acid monohydrate concentration in a sample solution (w/v\%)} = A \times \frac{Q_T}{Q_S} \times \frac{100}{B}$$

where A: Amount of citric acid monohydrate (g)

B: Volume of test sample (mL)

Test conditions:

Detector: Ultraviolet spectrophotometer (appropriate wavelength around 210nm)

Column: Use a suitable column that separates the peak of citric acid and an internal standard substance.

Column temperature, mobile phase and flow rate: Choose suitable conditions in consideration of the column used.

Calculate the sodium citrate content in a sample from the following formula. Correction is not made for test sample without free citric acid.

$$\text{Sodium citrate (dihydrate) content (w/v\%)} = [\text{the total citric acid (monohydrate) content} - D] \times 1.3995$$

where D: free citric acid (monohydrate) content (w/v%) of the sample determined by the citric acid quantitative method

## **Test for Mycobacterial Sterility**

### **Culture medium**

One percent Ogawa's medium shall be used, unless otherwise specified.

### **Volume of inoculum**

A 0.2-mL portion of the test sample shall be inoculated in each vessel of medium. Unless otherwise specified, each test sample shall be tested in more than 10 Ogawa's medium (1%) portions.

### **Culture and inspection**

The inoculated vessels shall be incubated at  $37 \pm 0.5^{\circ}\text{C}$  for no less than 6 weeks, unless otherwise specified.

### **Criterion for judgment**

No vessel shall show any evidence of the growth of mycobacteria.

## **Test for Optical Density**

The test for optical density is a method for determining the turbidity of a test sample by measuring the absorbance at 650 nm, unless otherwise specified, in a spectrophotometer. The criterion for judgment shall be given in the individual monographs.

### **Test Procedure**

A spectrophotometer shall be used. When the absorbance of water is adjusted to 0, the absorbance  $A$  of the reference opacity preparation should correspond to the opacity unit defined for the Reference. If necessary, a calibration curve may be drawn by plotting absorbances of a series of graded dilutions prepared accurately from the Reference in water for injection.

If the absorbance of the test sample is within the linear range of the calibration curve, the opacity unit of the test sample is estimated from the absorbance. If a diluted test sample is measured, the opacity unit shall be corrected by the diluting factor.

## **Test for Measuring the Potency of Human Anti-HBs Immunoglobulin**

The potency shall be determined by either “1. Radioimmunoassay” or “2. Enzyme immunoassay”. The criterion for judgment shall be given in the individual monographs.

### **Test procedures**

#### **1. Radioimmunoassay (RIA)**

Test sample and the National Standard anti-HBsAb (hereafter referred to as Standard) shall be subjected to accurately two-fold serial dilutions in several levels. The solid phase is coated with HBs antigen (Ag) by an appropriate method. Each dilution of the test sample or Standard shall be added to the solid phase together with iodine-125-labeled HBs Ag solution. After an appropriate period of incubation, the complex of antigen-antibody-antigen (iodine-125-labeled HBs Ag, anti-HBs antibody, and HBsAg in the solid phase) will be formed. The unbound tracer shall be washed away, and the residual radioactivity (bound to the solid phase) shall be measured with a gamma scintillation counter. The potency of test sample is calculated in consideration of the radioactivity of Standard as well as the dilution factor of test sample.

#### **2. Enzyme immunoassay (EIA)**

Test sample and Standard shall be subjected to accurately two-fold serial dilutions in several levels. The solid phase is coated with HBsAg by an appropriate method. Each dilution of the test sample or Standard shall be mixed with the solid phase together with the enzyme-conjugated HBsAg. After an appropriate period of incubation, a complex of enzyme-conjugated HBsAg, anti-HBs antibody and HBsAg in the solid phase will be formed. The unbound enzyme conjugates shall be washed away, and a chromogen substrate shall be added. After an appropriate period of incubation, the absorbance shall be measured colorimetrically with an EIA reader. The potency of test sample is calculated in consideration of the absorbance of Standard as well as the dilution factor of test sample.

## **Test for Measuring the Potency of Anti-D antibody**

The potency of anti-D antibody in a sample is assessed by using the indirect Coombs hemagglutination test. The criterion for judgment shall be given in the individual monographs.

### **Test Procedure**

Place 18 tubes (6 tubes x 3 rows) with a diameter of 7-8 mm. Prepare a series of two-fold dilutions from 500-to 16,000-fold of the test sample with physiological saline. Wash O type, Rho-positive red cells at least three times with physiological saline and suspend the packed cells as a 3% (v/v) suspension in physiological saline. Put 0.1 mL of a diluted test sample or saline (as a negative control) into a test tube. Then add 0.1 mL of the red cell suspension to each test tube. Mix the contents in each tube gently, and then incubate at 37°C for 30 minutes with occasionally shaking.

After washing the content in each test tube at least three times with physiological saline, add two drops of Coombs reagent. Mix the cell suspension thoroughly and then centrifuge for 1-2 minutes at  $190 \times g$ .

Suspend the cell pellet by gentle agitation and then grade the reaction visually in parallel with that of the negative control. The highest dilution that gives the minimum grade of hemagglutination is taken as the potency of the anti-D antibody.

## **Test for Freedom from Anti-complement Activity**

The test for freedom from anti-complement (AC) activity measures the residual AC activity when the defined amount of guinea pig complement is incubated with that test material. This test confirms that the test material would not inhibit the added complement activity more than the defined limit by binding to guinea pig complement.

### **Test procedure**

Add 1mL of 100 unit guinea pig complement to 1mL of test sample, then add and 3mL of buffer solution and mix well. After incubation at 37°C for 1 hour, dilute appropriately the incubated mixtures with the buffer solution. Add the defined volume of previously sensitized sheep red cells into the diluted solutions and incubate at 37°C for 1 hour. After centrifugating the reaction mixtures, measure the absorbance of the supernatants at 541 nm. As a complement control, perform the same procedure without test sample. Perform the statistical analysis to determine the residual complement activity, which is expressed as the 50% hemolytic complement dose (CH<sub>50</sub>).

A CH<sub>50</sub> unit is defined as the amount of complement that causes 50% hemolysis of 5×10<sup>8</sup> optimally sensitized sheep red blood cells at 37°C for 1 hour.

The amount of inactivated complement (AC) is expressed as follows.

$$AC = b - a$$

where a = the mean complement activity of the test sample

b = mean complement activity of complement control

(the b value must be no less than 85)

### **Criterion for judgment**

The value of AC in the test sample shall be no more than 20 unit (CH<sub>50</sub>).

## Test for Weight Variation

This test is to ensure the uniformity of content in final containers of injections intended to be dissolved or suspended for use.

### Test procedure

Select 10 container units, clean outside with water after removing labels, if necessary, to allow complete drying in a desiccator. Open carefully one of the containers and weigh accurately all parts of the opened container. Remove the contents of each container by washing sufficiently with water and ethanol and dry the contents completely in a desiccator. Weigh accurately all parts of the cleaned containers individually, and calculate for each unit the net weight of its contents by subtracting the weight of the container from its respective gross weight. Calculate the average weight of the ten containers and the deviation of weight of each container from the mean.

### Criterion for judgment

The requirements are met if not more than one of the number of products in a container shows a calculated deviation from the mean exceeding the percentage deviation in the table below and if the deviation is not greater than twice that specified in the table.

| Mean weight (g)       | Deviation (%) |
|-----------------------|---------------|
| $M < 0.015$           | 15            |
| $0.015 \leq M < 0.12$ | 10            |
| $0.12 \leq M < 0.3$   | 7.5           |
| $M \geq 0.3$          | 7             |

## **Cellulose Acetate Membrane Electrophoretic Test**

The cellulose acetate membrane electrophoretic test is a method to analyze the protein constituents in a test sample and to estimate their relative concentrations by differences in mobility of the protein solutions in electric fields.

Acceptance is assessed according to the criterion given in the individual monographs.

### **Test procedure**

The test sample is diluted with Sodium Diethylbarbiturate Buffer Solution (pH 8.6) to render the protein concentration to approximately 5% and then electrophoresed using a cellulose acetate membrane that has been equilibrated in the above buffer solution as support medium. After electrophoresis, the membrane is stained with Ponceau 3R. Protein constituents and relative concentrations are analyzed by densitometry.

## **Staining Test**

### **Test procedure**

Approximately 10 mL of the test sample is centrifuged in a pointed centrifuge tube at approximately  $2,000 \times g$  for 30 minutes. The sediment or the bottom portion is spread on a slide glass, dried and heat-fixed over a flame. The smear is then stained by the Gram's method and, unless otherwise specified, examined microscopically at an approximately 1,000-fold magnification.

### **Criterion for judgment**

No bacterial shall be observed other than those defined in the individual monographs.

## Test for Protein Content

Protein content is determined by measuring heated trichloroacetic acid-perceptible protein in the test sample by the Lowry method.

The test shall be done according to the following test procedure or other specified procedures. The criterion for judgment shall be given in the individual monographs.

### Test Procedure

Dissolve and dilute the standard albumin for protein determination with water accurately to make not fewer than three different concentrations of standard dilutions.

Dilute the sample with water to make a test dilution at a concentration in the range between the highest and lowest concentrations of the standard dilutions.

To each 1 mL of the test sample and standard dilutions taken accurately, add the same volume of 10 w/v% of trichloroacetic acid, and heat for 15 minutes in a boiling water bath. After cooling, centrifuge the mixture for 20 minutes at greater than  $1400 \times g$ . To the resulting precipitates, add 2 mL of 5 w/v% of trichloroacetic acid. Shake the mixture well, centrifuge again and remove the supernatant.

Add 2.5 mL of the Alkaline Copper Solution to the resulting precipitates, shake well and allow to stand for over 10 minutes. Add 2.5 mL of water and 0.5 mL of the diluted Folin's test solution to the solution and keep standing for 30 minutes at  $37^{\circ}\text{C}$ . Measure the absorbance of the solution (supernatant collected by centrifugation for 20 minutes at over  $1400 \times g$  if the solution is turbid) at 750 nm with a spectrophotometer.

Determine the protein content of the test dilution from the calibration curve obtained from the standard solutions and calculate the protein content per mL of the test sample.

Correct the value by measuring the absorbance of water treated in the same manner as above.

## Test for Protein Nitrogen Content

The protein nitrogen content test is a method used to determine protein content by measuring nitrogen in heated trichloroacetic acid-precipitable protein in the test sample by the micro-Kjeldahl method. The criterion for judgment shall be given in the individual monographs.

### Test procedure

Dilute test sample with water, if necessary, and transfer a suitable quantity to a centrifuge tube. Add a one-tenth volume of a 50 w/v% trichloroacetic acid solution to render trichloroacetic acid concentration to 4.5 w/v% or higher.

Heat the mixture at 100°C for 15 minutes and allow to cool to room temperature. With regard to antitoxins, therapeutic sera, and blood products listed in the monographs, this heat treatment is replaced by warming at an appropriate temperature for 15 minutes. Centrifuge the mixture at greater than  $1400 \times g$  for 10 minutes. Add an appropriate amount of a 5 w/v% trichloroacetic acid solution to the precipitate, shake and centrifuge again. Measure the nitrogen content in the precipitate using an appropriate method such as the micro-Kjeldahl method.

Calculation of protein content;

The protein content is calculated from the nitrogen content by the following formula:

$$1 \text{ mg protein nitrogen (N)} = 6.25 \text{ mg protein}$$

## Test for Thimerosal Content

The test for thimerosal content consists of “1. Chemical determination of total thimerosal content”, “2. Atomic absorption spectrophotometry (reduction cold vapor)” or “3. Atomic absorption spectrophotometry (direct combustion/catalyst gold trap)”. The criterion for judgment shall be given in the individual monographs.

### 1. Chemical determination of thimerosal content

The method for chemical determination of thimerosal content is based on the fact that thimerosal reacts with dithizone, resulting in formation of a compound having maximum specific absorption at 480 nm.

#### Test procedures

Dilute the 0.02 w/v% thimerosal standard accurately with water to make not fewer than three different concentrations of standard dilutions.

Dilute the sample in water to make a test dilution at a concentration in the range between the highest and lowest concentrations of standard dilutions.

To each 0.5 mL of the test sample and standard dilutions taken accurately, add 4.5 mL of water. Add 5 mL of dilute sulfuric acid and 10 mL of the dithizone test solution, shake for 5 minutes and allow to stand. Collect 5 mL of the resulting carbon tetrachloride layer (if necessary, centrifuged at over  $1,400 \times g$  for 10 minutes to obtain better separation), shake with 10 mL water and allow to stand. Discard the resulting water layer, shake the rest with 10 mL of the ammonia test solution (9 mol/L), and allow to stand and discard the water layer. Repeat this procedure of washing with the ammonia test solution three times; then add 10 mL of water, shake and allow to stand. Discard the resulting water layer and filter the remaining carbon tetrachloride layer through a filter paper. Measure the absorbance of the filtrate at 480 nm with a spectrophotometer.

Correct the value by measuring the absorbance of water treated in the same manner as above.

Determine the thimerosal content of the test dilution from the calibration curve obtained from the standard dilutions.

### 2. Atomic absorption spectrophotometry (reduction cold vapor)

Use the following method or other equivalent method.

Cold vapor atomic absorption spectrophotometry consists of the following steps: 1. digestion of a sample with potassium permanganate; 2. reduction of mercury in the sample with stannous chloride solution; and 3. measurement of atomic absorption at 253.7 nm of evolved mercury vapor from the solution by aeration and quantitative determination of mercury content.

#### Test procedure

Dilute a 0.02 w/v% thimerosal standard solution accurately with water to make not fewer than three different concentrations of standard dilution. Dilute the sample with water to make a test dilution at a concentration in the range between the highest and lowest concentrations of standard dilutions.

According to the JIS K0102 industrial drainage examination method (reduction cold vapor atomic absorption spectrophotometry) or the corresponding JIS K0101 water-for-industrial-use examination

method (reduction cold vapor atomic spectrophotometry), transfer the proper amount of the standard dilutions and a sample to glass vessels, and add water to make approximately three-fifths of the final sample volume. To one volume of test sample, add a 1/12.5 volume of diluted sulfuric acid (1→2), a 1/50 volume of nitric acid and a 1/12.5 volume of potassium permanganate solution (1→20), and mix. Allow the mixture to stand for about 15 minutes. Add a 1/25 volume of potassium persulfate solution (1→20) and heat the mixture for 2 hours at approximately 95°C. Cool the mixture to a room temperature, add a 1/25 volume of hydroxylamine hydrochloride solution (8→100), and add water to make the final sample volume. Add a 1/25 volume of stannous chloride solution to the solution and measure.

Calculate the thimerosal content in a sample from the calibration curve obtained from the measurement of the standard dilutions.

### **3. Atomic absorption spectrophotometry (direct combustion/catalyst gold trap)**

Use the following method or other equivalent method.

This method consists of the following steps: 1. direct sample combustion at 700–1000°C; 2. transformation to gaseous mercury with a catalyst; 3. collection and evolution of all mercury with a gold trap; and 4. determination of absorbance at 253.7 nm with a spectrophotometer

#### **Test procedure**

Dilute a 0.02 w/v% thimerosal standard solution accurately with water to make not fewer than three different concentrations of standard dilution. Dilute the sample with water to make a test dilution at a concentration in the range between the highest and lowest concentrations of the standard dilutions.

To a ceramic combustion boat (80 mm × 15 mm) filled and covered with alumina granules (approximately 1 mm diameter) to 1/4 volume of the boat, charge 0.1 mL of the standard dilutions or a sample solution accurately. Cover with the alumina granules (additional 1/4 volume of the boat). Fill up and covers the boat with a half volume of additive agent (calcium hydroxide and sodium carbonate mixture, volume ratio = 1:1).

Start the measurement at 350°C for 4 minutes, and then at 700°C for 6 minutes.

Calculate the thimerosal content in a sample from the calibration curve obtained from the measurement of standard dilutions.

## Test for Sugar Content

Carbohydrate content is determined by either “1. Absorption spectrophotometry” or “2. Liquid chromatography”.

### 1. Absorption spectrophotometry

Sugars in a test sample (lactose and glucose) are converted into reducing sugar, which is then reacted with anthrone. The sugar content is determined by measuring the absorbance of the resulting product at 620 nm.

#### Test procedure

Dilute a 0.01 w/v% glucose standard or a 0.01 w/v% lactose standard solution with water accurately to make not fewer than three different concentrations of standard dilutions. Dilute the sample with water to make a test dilution at a concentration in the range between the highest and lowest concentrations of the standard dilutions. To each mL of the test sample and the standard dilutions taken accurately, add 10 mL of anthrone sulfuric acid test solution, shake well, and heat for 15 minutes at 100°C. Cool in ice water and allow to stand for 30–60 minutes at room temperature. Measure the absorbance of the mixtures at 620 nm with a spectrophotometer.

Determine the reduced carbohydrate content of the test dilution from the calibration curve obtained from the standard dilutions and calculate the carbohydrate (lactose or glucose) content of the test sample.

Correct by measuring the absorbance of water treated in a same manner.

### 2. Liquid chromatography (HPLC)

This method determines the carbohydrate content (lactose or glucose) in a sample quantitatively by liquid chromatography (HPLC).

#### Test procedure

Take an appropriate amount of test sample and remove the protein the sample contains by an appropriate method if necessary. Prepare a test sample solution by adding a fixed amount of the internal standard solution and water if necessary to the test sample. Transfer an appropriate amount of carbohydrate (lactose or glucose) accurately and prepare a standard solution in the same manner as the test sample solution. Transfer a fixed amount of the test sample solution and the standard solution and submit to liquid chromatography according to the liquid chromatography method of General Tests, Processes and Apparatus in the JP. Determine the ratio of the peak area of carbohydrate (lactose or glucose) to that of the internal standard in the test sample solution ( $Q_T$ ) and in the standard solution ( $Q_S$ ). Calculate the total amount of carbohydrate (lactose or glucose) in the sample by the following formula.

$$\text{Carbohydrate (lactose or glucose) content (w/v\%)} = A \times Q_T/Q_S \times B/100$$

where A: carbohydrate (lactose or glucose) used (g)

B: test sample used (mL)

Test conditions;

Detector: Differential refractive index detector

Column: Use a suitable column that separates each peak of the carbohydrate (lactose or glucose) and the internal standard substance completely.

Column temperature, mobile phase, and flow rate: Choose suitable conditions in consideration of the column used.

## **Test for Sodium Content**

Sodium content is determined with a flame spectrophotometer or an atomic absorption spectrophotometer. The criterion for judgment shall be given in the individual monographs.

### **Test procedure**

Transfer a suitable quantity of test sample and dilute it with water or an appropriate diluent, if necessary. Measure the brightness at 589 nm in a flame spectrophotometer or the absorbance at 589 nm in an atomic absorption spectrophotometer of test material.

Dilute sodium standard dilutions accurately to make not fewer than three different concentrations of standard dilutions. Dilute the sample accurately to make a test dilution at a concentration in the range between the highest and lowest concentrations of the diluted standard solutions. Determine the brightness or absorbance of the test solution and standard dilutions. Calculate the sodium content in the test sample from the calibration curve obtained from the standard dilutions.

## **Test for Heat Stability**

Test for Heat Stability is for ensuring the stability of protein under heat treatment.

### **Test procedure**

Transfer 2 mL of a sample into a test tube (75 mm length, 12 mm inner diameter) with a cap, heat at 57°C for 4 hours and observe macroscopically immediately.

### **Criterion for judgment**

Gel formation in the sample solution shall not be observed after heat treatment.

## **Test for Tetanus Antitoxin Titer Determination**

The Tetanus Antitoxin Titer Determination method is a method for measuring tetanus antitoxin titer in a test sample by using the toxin neutralization reaction, unless otherwise stated. The criterion for judgment shall be given in the individual monographs.

### **Test Procedure**

The test sample, Standard Tetanus Antitoxin or Standard Anti-tetanus Human Immunoglobulin and Tetanus Test Toxin shall be used. Dilution of these materials shall be made in a 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) containing 0.2 w/v% gelatin.

The Standard Tetanus Antitoxin or Standard Anti-tetanus Human Immunoglobulin shall be diluted to five levels at appropriate intervals determined with careful consideration to the accuracy of the test and contain 0.1 units per 0.2 mL in the median dilution (hereafter referred to as "standard dilution" in this monograph). If the antitoxin titer in the test samples shows a tendency to relatively stability, three levels of dilution containing 0.09, 0.10, 0.11 international units may be applied. The test sample shall be serially diluted in similar intervals (hereafter referred to as the "test dilution" in this monograph).

The test toxin shall be diluted to contain one test dose per 0.2 mL (hereafter referred to as the "toxin dilution" in this monograph).

A volume shall be taken accurately from each of the standard and test dilutions, combined with an equal volume of the corresponding toxin dilution and mixed well. Each mixture shall be kept standing for 1 hour and then injected subcutaneously at a dose of 0.4 mL into the inguocrural region of at least 4 mice aged 23-29 days. The animals shall be observed for 5 days after injection.

Antitoxin content of the test sample shall be determined by statistical analysis of test results.

## Pyrogen Test

The pyrogen test is a method to estimate the pyrogenic activity of a test sample based on the febrile response of rabbits to intravenous injection of a test sample.

### Animals

Rabbits weighing not less than 1.5 kg are used. Rabbits that have been used for the test may be used again 3 days or longer after the previous use in the test, excluding those belonging to any group showing a positive response in the previous test, or a group inoculated with a material containing the same antigenic substances contained in the test sample.

Rabbits shall be kept in a room at 20–27°C with as uniform a temperature and humidity as possible for 2 days or more prior to the start of the test until the completion of the test.

### Instruments

The instrument used to measure body temperature shall provide accurate grading to 0.1°C.

Syringes and needles shall be heated at 250°C for not less than 30 minutes prior to the test. When plastic apparatus, such as plastic syringes, are used, they shall be free of detectable pyrogens and shown not to interfere with the test.

### Test procedure

#### 1) Dose

Unless otherwise specified in individual monographs, the test dose per kg body weight shall be 3.0 mL.

#### 2) Method

Withhold food from the rabbits for several hours prior to the injection and until the test is completed. The animals are not to be restrained too tightly.

A thermometer or other recording device is inserted into the rectum to a constant depth within the range of 60 to 90 mm. The temperature is read after the elapse of sufficient time after insertion.

The “initial” temperature of each rabbit is determined prior to the injection of the test sample. All rabbits having an initial temperature higher than 39.8°C are to be withdrawn from the test. The test sample shall be warmed to approximately 37°C and injected into the ear vein within approximately 15 minutes after the determination of the initial temperature. In general, temperatures are recorded at regular intervals of up to 1 hour for 3 hours after injection.

The highest temperature recorded in a rabbit during the 3 hours after injection is regarded as the “maximum” temperature. The difference between the initial temperature and the maximum temperature is regarded as its response. When the difference is negative, the response is interpreted as “0”.

### Criterion for judgment

The test shall be carried out first on a group of three rabbits and the results shall be judged from the following table. If the summed response of the group lies between the values given in the third and fourth columns of the table, the test shall be repeated on another group of three rabbits, and the summed response of the six rabbits is then judged from the table. No more than three groups are used with a test sample as seen from the table.

| Number of experiments | Cumulative number of rabbits | Test sample passes if the summed response is | Test sample fails to pass if the summed response is |
|-----------------------|------------------------------|--|---|
| 1                     | 3                            | 1.3°C or less                                | 2.5°C or more                                       |
| 2                     | 6                            | 3.0°C or less                                | 4.2°C or more                                       |
| 3                     | 9                            | less than 5.0°C                              | 5.0°C or more                                       |

### **Test for pH**

pH is determined by the pH determination method specified in General Tests, Processes and Apparatus in the JP. The criterion for judgment shall be given in the individual monographs.

## Test for Phenol Content

Phenol content is determined by measuring the color developed by the reaction with the p-nitroaniline and nitrous acid test solution.

### Test procedure

Transfer a 1-mL portion of the test sample accurately and add water to make a 50-mL test dilution. In the case of antitoxins, transfer a 1-mL portion accurately, add 10 mL of water and 10 mL of 5 w/v% trichloroacetic acid solution, and make up to a 50-mL solution by adding water.

Allow the dilution to stand at room temperature for 30 minutes and filter. Use the filtrate as test dilution.

Treat a 1-mL portion of a 0.5 w/v% phenol standard solution in the same manner as the test sample and use as standard dilution. Take 1-mL portions of the test dilution and standard dilution accurately and add approximately 30 mL of water. To each dilution solution, add 1 mL of a 50 w/v% sodium acetate solution and then 1 mL of p-nitroaniline-sodium nitrite test solution and shake well. Add 2 mL of the sodium carbonate test solution and make up accurately to 50 mL by adding water, and then shake well. Allow to stand at room temperature for 10 minutes. Measure a portion of the mixture to measure absorbance at 480 nm.

Determine the phenol content in the test sample from the absorbance values of the test dilution and the standard dilutions.

Correct by measuring the absorbance of water treated in the same manner as the test sample.

### Criterion for judgment

The phenol content in the test sample shall be between 0.45 and 0.55 w/v%, unless otherwise specified in the individual monographs.

## **Test for Heme Content**

Heme content is determined by measuring absorbance of the test sample at 403 nm.

### **Test procedure**

Measure the absorbance of the test sample at 403 nm with an appropriate spectrophotometer having a light path of 10 mm.

### **Criterion for judgment**

The absorbance shall be no more than 0.25.

## **Test for Hemoglobin Content**

Hemoglobin content is determined by measuring the color of cyanmethemoglobin developed using van Kampen's test solution (pH 7.2). The criterion for judgment shall be given in the individual monographs.

### **Test procedure**

Transfer a 0.02-mL portion of test material or of a test sample prepared by diluting the test material with water, if necessary, and add 5 mL of van Kampen's test solution (pH 7.2). Allow to stand at approximately 25°C for 5 minutes and measure the absorbance at 540nm with a spectrophotometer.

Prepare a dilution series of cyanmethemoglobin standard solution covering several suitable concentrations by accurately diluting with water, measure the absorbance of each of the dilutions at 540 nm and calculate the hemoglobin content of the test material from the calibration curve obtained from the standard dilutions.

### **Test for Formaldehyde Content**

Formaldehyde content is determined by measuring the intensity of the color of 3,5-diacetyl-1,4-dihydroxylutidine resulting from the reaction with acetylacetone in the presence of excess ammonium salt under slightly acidic conditions. The criterion for judgment shall be given in the individual monographs.

#### **Test procedure**

Dilute the 0.04 w/v% formaldehyde standard solution accurately with water to make not fewer than three different concentrations of standard dilutions. Dilute the sample with water to make a test dilution at a concentration in the range between the highest and lowest concentrations of standard dilutions.

To each 5 mL of the test sample and the standard dilutions taken accurately, add 5 mL of the acetylacetone solution, heat for 15 minutes in a boiling water bath, and then cool. For solutions which are turbid, centrifuge the mixture for 20 minutes at over  $1400 \times g$  and transfer the supernatant. Measure the absorbance at 415 nm and determine the formaldehyde content in the test dilution from the calibration curve obtained from the standard dilutions

Correct by measuring the absorbance of water treated in the same manner as the test sample.

## **Mycoplasma Test**

Mycoplasma Test is a method to establish the absence of detectable mycoplasmas in samples using following tests, unless otherwise specified.

### **1. Culture media**

Agar plate medium for mycoplasma testing (hereafter referred to as "agar medium plate" in this monograph) and broth medium for mycoplasma testing I and II are used, unless otherwise specified.

### **2. Product sampling**

Live vaccines shall be tested before the filtration step of the manufacturing process. Inactivated vaccines shall be tested before the inactivation step. The volume of the test samples is 6mL. Samples shall be stored at a temperature between 2°C and 8°C if the test is performed within 24 hours of obtaining the sample. If more than 24 hours elapses before the test is performed, the samples shall be stored at -60°C or less.

### **3. Culture and observation**

Both agar and broth medium shall be used.

#### **3.1 Agar cultures**

Inoculate a 0.2-mL portion of the test sample over the surface of each of ten or more agar plates. After the inoculation, the surfaces of the plates are dried and the plates are incubated in an air containing 5 to 10 vol% carbon dioxide or nitrogen atmosphere at 35–37°C.

#### **3.2 Broth cultures**

Inoculate a 0.2-mL portion of the test sample into each of 10 or more test tubes containing 10 mL of broth medium for mycoplasma testing I and also into same number of test tubes containing 10 mL of broth medium for mycoplasma testing II. After inoculation, test tubes are incubated at 35–37°C for not less than 14 days. A 0.2-mL portion of each culture from the fifth to seventh incubation days and from the last incubation day are subcultured in corresponding fresh broth medium for not less than 14 days at the same temperature. Original cultures continue to be incubated for the same period. If a color change is observed in any of the broth media, the medium shall be diluted 10-fold serially with physiological saline. A 0.1-mL portion of each dilution is inoculated on two or more agar plates and incubated as indicated in 3.1.

### **4. Observation**

Color change in broth medium is checked during the culture period. Each agar plate is incubated for not less than 14 days and observed for the presence of colonies on the seventh and last incubation day. If suspected colonies are found, they shall be observed microscopically after staining with Dienes solution.

### **5. Validation tests for growth-inhibiting factors**

Prior to mycoplasma testing, the samples shall be tested to detect the presence of any factors

that inhibit mycoplasma growth. *Acholeplasma laidlawii* is used as the standard organism for the detection of such growth-inhibiting activity. However, if another mycoplasma strain is known to be more susceptible to the growth-inhibiting factor, that strain shall be used instead of *Acholeplasma laidlawii*. The broth medium for mycoplasma testing I is used for tests employing *Acholeplasma laidlawii* or the other glucose-metabolizing mycoplasmas. The broth medium for mycoplasma testing II is used for tests with arginine-metabolizing mycoplasmas. Inoculate approximately 100 CFU of mycoplasmas into the broth medium containing test sample with the volume specified in 3.2 and incubate at 35–37°C for 7 days. Observe for the occurrence of any color change in the medium. If the growth of mycoplasmas is not observed, or is poor, it is considered that the test sample possesses growth-inhibiting activity. In this case, use a suitable inactivating agent which does not affect the growth of mycoplasmas or increase the volume of medium while maintaining the volume of the test sample, irrespective of the description in 3.2 so that no growth-inhibiting activity remains. For products that have strong growth-inhibiting activity, the membrane filtration method specified below can be applied. Taking these steps to eliminate growth-inhibiting activity, the products are retested and the validity of the testing methods is confirmed. Once a batch of medicine has been tested, medicine subsequently produced through the same process does not require per-batch testing.

#### **6. Membrane filtration method**

The test sample is filtered with a 0.1 µm pore sized membrane filter, if necessary with rinsing of the membrane three times with 10 mL of phosphate buffer (pH 7.2). The processed membrane is aseptically transferred from the apparatus and cut into two equal parts. Alternatively, the membrane is cut into two equal parts prior to the filtration and processed in a similar manner. The pieces of the membrane thus prepared are inoculated into 100 mL of the broth medium for mycoplasma testing I and II. Subsequent culture and subculture are performed according to the procedure described in 3.2.

#### **7. Criterion for judgment**

If no evidence for the growth of mycoplasmas is found as a result of the above-mentioned test, the product tested is deemed to meet the requirements for mycoplasma testing.

## Antibody Assay against Measles Virus

Antibody Assays against Measles Virus is carried out by the following three methods;

1. Neutralization test
2. Hemagglutination inhibition test
3. Passive hemagglutination test

The criterion for judgment shall be given in the individual monographs.

### 1. Neutralization test

Prepare serial two-fold dilutions of the samples and reference antiserum for measles with appropriate diluents. Add 1mL of virus solution containing 100 CCID<sub>50</sub>/0.1 to 1 mL of each the sample or reference antiserum. Mixed well and incubate at 2–8°C for 1 hour or at 37°C for 30 min. Then inoculate 0.2 mL of each mixture into measles virus-sensitive cells grown in several culture tubes. After incubating at 35–37°C for 7–8 days, observe the appearance of CPE of the cells in each tube by microscopy. Statistically analyze the results to calculate the neutralization titer (highest dilution of sera which cause 50% inhibition) of the samples and reference antisera.

Antibody titers of samples are calculated by the following formula:

$$\text{Antibody titer of samples} = \text{antibody titer of reference antiserum for measles} \times \frac{\text{neutralizing titer of sample}}{\text{neutralizing titer of reference antiserum}}$$

### 2. Hemagglutination inhibition (HI) test

Collect 0.1-mL portions each of samples and reference serum, and add 0.3 mL of PBS (0.067mol/L, pH7.2) and 0.4mL of Kaolin solution into each tube. Incubate at room temperature for 20 minutes with occasional shaking. Then centrifuge the tubes at 760 g for 10 minutes, and collect the supernatant of each tube. Add 0.1mL of 50 vol% of green monkey red blood cells into tubes, and incubate at room temperature for 1 hour with occasional shaking. Then centrifuge the tubes at 760 × g for 10 minutes, and collect the supernatants. These supernatant solutions are defined as the samples and reference sera diluted 1:8.

Subsequently, dilute again these 8-fold diluted samples with 0.1 w/v% albumin, PBS (0.067 mol/L, pH 7.2) containing 0.01% gelatin (?PBS containing 0.1 w/v% albumin and 0.01% gelatin) a further 5-, 7-, 40-, and 56-fold (diluted samples).

Further, dilute again these 8-fold diluted reference sera with 0.1 w/v% albumin, PBS (0.067 mol/L, pH 7.2) containing 0.01% gelatin a further 5- and 7-fold (diluted reference sera).

Collect 25 µl of the diluted samples and reference sera and place into the wells of microplates. After two-fold dilution of these samples and reference sera, add 25 µl of measles antigen (4 units) into each wells, and shake well. After incubating them at room temperature for 1 hour, add 50 µl of 0.5 vol% of green monkey red blood cells suspension, and shake well. Incubate at 37°C for 2 hours, then read the results of hemagglutination macroscopically.

The highest dilution of sample and reference sera that causes the inhibition of hemagglutination is considered the HI titration endpoint.

Antibody titers of samples are calculated by the following formula:

Antibody titer of samples = antibody titer of reference antiserum for measles × HI titer of sample/HI titer of reference antiserum

### **3. Passive hemagglutination (PHA) test**

Dilute samples and reference sera with appropriate buffer solution to make 1:160 and 1:224 dilutions, respectively. Transfer 25 µl of these diluted samples and reference sera into the wells of microplates. Then prepare two-fold dilutions of these diluted samples and reference sera with appropriate buffer solution. Add 25 µl of appropriate buffer solution and 25 µl of sheep RBC sensitized with measles antigen into each well. After shaking well incubate the plates at room temperature for 3 hours, then read the results of hemagglutination macroscopically.

The highest dilution of sample and reference sera that causes hemagglutination is considered the PHA titration endpoint.

Antibody titers of samples are calculated by the following formula:

Antibody titer of samples = antibody titer of reference antiserum for measles × PHA titer of sample/PHA titer of reference antiserum

## Sterility Test

The Sterility Test is a method to establish the presence or absence of viable microorganisms (bacteria or fungi) using a defined cultivation method. Unless otherwise specified, the test is carried out by the procedure set forth in Sterility Test under “General Tests, Processes and Apparatus” in the JP. For tests carried out on final bulk or earlier stages of manufacture, the quantities of the material to be collected and inoculated into each medium shall not be less than those indicated in Table 1, unless otherwise specified.

Table 1

| Minimum sample quantity | Medium                       | Quantity of inoculum for each medium |  |
|-------------------------|------------------------------|--------------------------------------|--|
|                         |                              | Membrane filtration                  | Direct inoculation   |
| 20 mL                   | Fluid thioglycollate medium  | 10 mL                                | 1 mL each for 8 test vessels<br>and<br>0.5 mL each for 8 additional test vessels |
|                         | Soybean-casein-digest medium | 10 mL                                | 1 mL each for 8 test vessels   |

## Test for Sodium Dihydrogenphosphate Content

Sodium dihydrogenphosphate content is determined by measuring the molybdenum blue (blue color) resulting from the reduction of phosphomolybdic acid produced from the reaction of sodium dihydrogenphosphate in sample with molybdenum acid in a sulfuric acid solution. The criterion for judgment shall be given in the individual monographs.

### Test procedure

Transfer a 5-mL portion of the test sample accurately, and add water to make a 100-mL test dilution. To the 5-mL sample solution taken accurately in a 25-mL measuring flask, add 10 mL of 0.5 mol/L sulfuric acid test solution, 2.0 mL of 2.5-w/v% ammonium molybdate solution, and 1.0 mL of 1-amino-2-naphthol-4-sulfonic acid test solution successively with mixing. Dilute the mixture with water to make a 25-mL solution accurately and allow to stand at 20–25°C for 15 minutes.

Measure the absorbance at 760 nm by a spectrophotometer within 5 minutes.

Simultaneously, measure the absorbance of 5-mL phosphoric acid standard solution prepared in the same manner as the test sample.

Correct by a blank test using water prepared in the same manner as the test sample.

The sodium dihydrogenphosphate content in a sample is calculated by the following formula where S, T and C are the absorbance of the phosphoric standard solution, the corrected absorbance of the sample solution, and the concentration of the potassium dihydrogenphosphate in the phosphoric standard solution, respectively.

$$\text{Sodium dihydrogenphosphate dihydrate (mg/mL)} = 22.93 \times C \times \frac{T}{S}$$

## **B. Standards, Reference Preparations, Test Toxins and Units**

National Standards and National Reference Preparations are specified products to be used as measures for the potency or toxicity of a product. These preparations are intended for use only in laboratory tests and do not necessarily meet the requirements for the product to be tested. The specified biological activity exhibited by a defined amount of each National Standard is defined as one unit. Units are usually not defined for National Reference Preparations.

International Standards and Reference Preparations are certain preparations authorized by the World Health Organization. A unit of a specified biological International Standard and Reference Preparation shall be used for standardizing National Standards or Reference Preparations, respectively.

When the specified biological activity of a National Standard is expressed in terms of units, International Units shall be used. In the case of National Standards for which no International Unit has been assigned, specific biological activity shall be expressed in terms of National Units.

### **1. National Standards and Reference Preparations**

#### **1.1 Antigens**

Standard Influenza Vaccine (for CCA test)

A liquid product containing 1,000 CCA of inactivated influenza virus per mL

Reference Influenza Vaccine (for neutralization test in eggs)

A liquid product containing inactivated influenza virus derived from specified strains at concentrations specified on the label

Reference Influenza Vaccine (for decrease in leukocyte count examination in mice)

A dried product containing purified and inactivated influenza virus derived from specified strains at concentrations specified on the label

Standard Influenza HA Antigen (for a single radial immunodiffusion test)

A dried product containing inactivated influenza HA antigen derived from specified strains at concentrations specified on the label

Reference Influenza HA Vaccine (for neutralization test in eggs)

A liquid product containing influenza HA vaccine derived from specified strains at concentrations specified on the label

Reference Hepatitis A Vaccine (for potency test)

A dried product containing inactivated hepatitis A virus at a specified dose. It shall be dissolved with water for injection and diluted with physiological saline to 1 reference unit per mL when used in laboratory tests.

Reference HBs Ag (for purity test)

A liquid product containing HBs Ag at a concentration specified on the label

Reference Inactivated Rabies Vaccine

A liquid product containing inactivated rabies virus derived from a specified strain at a concentration specified on the label

Reference Cholera Vaccine (Ogawa serotype)

A dried product containing inactivated cholera vibrios of the Ogawa serotype at a specified concentration. To use in the test, it shall be reconstituted with physiological saline.

The amount of the reconstituent to be added is given on the label.

Reference Cholera Vaccine (Inaba serotype)

A dried product containing inactivated cholera vibrios of Inaba serotype at a specified concentration. To use in the test, it shall be reconstituted with physiological saline. The amount of the reconstituent to be added is given on the label.

Standard Diphtheria Toxoid

A dried product containing "diphtheria toxoid" at a concentration specified on the label. To use in the test, 0.017 M phosphate-buffered sodium chloride solution containing 0.02% (w/v) gelatin (pH 7.0) is added as the reconstituent.

Standard Adsorbed Diphtheria Toxoid

A dried product containing "diphtheria toxoid" and aluminium hydroxide at a specified concentration. To use in the test, physiological saline is added as the reconstituent.

Reference Diphtheria Toxoid (for Combined Vaccine)

A dried product containing "diphtheria toxoid", inactivated pertussis bacilli components and tetanus toxoid at a specified concentration. To use in the test, physiological saline is added as the reconstituent.

Reference Adsorbed Diphtheria Toxoid (for Combined Vaccine)

A dried product containing "diphtheria toxoid", purified pertussis vaccine, tetanus toxoid and aluminium hydroxide at a specified concentration. To use in the test, physiological saline is added as the reconstituent.

Standard Purified Tuberculin

A dried product containing 50,000 IU of purified tuberculin (PPD) per mg. It shall be reconstituted with a reconstituent specified on the label and used immediately after reconstitution.

Reference Smallpox Vaccine

A dried product containing live vaccinia virus, of which the pock-forming units is stated on the label. It shall be reconstituted with a reconstituent specified on the label and used immediately after reconstitution.

Reference Smallpox Vaccine Prepared in Cell Cultures (LC16m8 strain)

A dried product containing live vaccinia virus (LC16m8 strain), of which the potency in terms of pock-forming units is stated on the label. It shall be reconstituted with a reconstituent specified on the label and used immediately after reconstitution

Reference Japanese Encephalitis Vaccine

A dried product containing inactivated Japanese encephalitis virus derived from a specified strain at a specified concentration. To use in the test, it shall be reconstituted with physiological saline. The amount of reconstituent to be added is given on the label.

Standard Tetanus Toxoid

A dried product containing "tetanus toxoid" at a specified concentration. To use in the test, 0.017 mol/L phosphate-buffered sodium chloride solution containing 0.02% (w/v) gelatin (pH 7.0) is added as the reconstituent.

#### Standard Adsorbed Tetanus Toxoid

A dried product containing "tetanus toxoid" and aluminium hydroxide at a specified concentration. To use in the test, isotonic physiological saline is added as the reconstituent.

#### Reference Tetanus Toxoid (for Combined Vaccine)

A dried product containing "tetanus toxoid", inactivated pertussis bacilli components and diphtheria toxoid at a specified concentration. To use in the test, physiological saline is added as the reconstituent.

#### Reference Adsorbed Tetanus Toxoid (for Combined Vaccine)

A dried product containing "tetanus toxoid", purified pertussis vaccine, diphtheria toxoid and aluminium hydroxide at a specified concentration. To use in the test, physiological saline is added as the reconstituent.

#### Reference Adsorbed Habu Toxoid

A dried product containing "Habu toxoid" and aluminium hydroxide at a specified concentration. To use in the test, physiological saline is added as the reconstituent.

#### Reference Hepatitis B Vaccine (for potency test)

A liquid product containing inactivated HBs Ag made insoluble by the addition of aluminium hydroxide. To use in the test, it shall be diluted with physiological saline to one reference unit per mL.

#### Standard Pertussis Vaccine

A dried product containing inactivated pertussis bacilli at a specified concentration and corresponding to 51 IU per ampoule. It shall be used as the unit for potency testing of the adsorbed Pertussis Vaccine. It shall be reconstituted with a reconstituent specified on the label.

#### Reference Pertussis Vaccine (for Toxicity tests)

A dried product containing inactivated pertussis bacilli corresponding to 36 LPU, 48 HSU and 1,368 BWDU per ampoule.

#### Reference Leptospira Interrogans Serogroup Autumnalis Combined Vaccine

A dried product containing inactivated Weil's leptospira, leptospira interrogans serogroup autumnalis A, B and C at a specified concentration. To use in the test, autoclaved distilled water is added as the reconstituent.

#### Reference Weil's Leptospira (for Agglutination Test)

A dried product containing Weil's leptospira (Copenhageni). To use in the test, it shall be incubated with Kolthoff medium or a suitable equivalent culture medium, and used it at a specified concentration.

#### Reference Leptospira Interrogans Serogroup Autumnalis A (for Agglutination Test)

A dried product containing Leptospira Interrogans Serogroup Autumnalis A (Autumnalis). To use in the test, it shall be incubated with Kolthoff medium or suitable equivalent culture medium, and used it at a specified concentration.

Reference *Leptospira Interrogans* Serogroup Autumnalis B (for Agglutination Test)

A dried product containing *Leptospira Interrogans* Serogroup Autumnalis B (Hebdomadis). To use in the test, it shall be incubated with Kolthoff medium or suitable equivalent culture medium, and used it at a specified concentration.

Reference *Leptospira Interrogans* Serogroup Autumnalis C (for Agglutination Test)

A dried product containing *Leptospira Interrogans* Serogroup Autumnalis C (Australis). To use in the test, it shall be incubated with Kolthoff medium or suitable equivalent culture medium, and used it at a specified concentration.

Reference Weil's *Leptospira* (for Aggression Test)

A liquid product containing Weil's *Leptospira* (*Copenhageni*) which is toxic in guinea pig

## 1.2 Antibodies

Reference Influenza HA antiserum

An antiserum in rabbit, guinea pig or goat immunized with inactivated influenza HA antigen derived from a specific strain.

Standard Gas Gangrene Antitoxin (*Clostridium perfringens* Type A)

A dried product containing "gas gangrene antitoxin (*C. perfringens* Type A)." To use in the test, physiological saline is added as the reconstituent.

Standard Gas Gangrene Antitoxin (*Clostridium septicum*)

A dried product containing "gas gangrene antitoxin (*C. septicum*)."

To use in the test, physiological saline is added as the reconstituent.

Standard Gas Gangrene Antitoxin (*Clostridium oedematiens*)

A dried product containing "gas gangrene antitoxin (*C. oedematiens*)."

To use in the test, physiological saline is added as the reconstituent.

Standard Diphtheria Antitoxin

A dried product containing "diphtheria antitoxin." To use in the test, physiological saline is added as the reconstituent.

Reference Diphtheria Antitoxin (for flocculation test)

A dried product containing "diphtheria antitoxin." To use in the test, physiological saline is added as the reconstituent.

Reference Anti-tetanus Human Immunoglobulin

A liquid product containing labeled IU of "anti-tetanus antibody" per vial.

Standard Tetanus Antitoxin

A dried product containing "tetanus antitoxin." To use in the test, physiological saline is added as the reconstituent.

Reference Tetanus Antitoxin (for flocculation test)

A dried product containing "tetanus antitoxin." To use in the test, physiological saline is added as the reconstituent.

Standard Habu (*Trimeresurus flavoviridis*) Antivenin

A dried product containing "Habu antivenins." To use in the test, physiological saline is added as the reconstituent

#### Standard Botulism Type A Antitoxin

A dried product containing "botulism type A antitoxin." To use in the test, physiological saline is added as the reconstituent.

#### Standard Botulism Type B Antitoxin

A dried product containing "botulism type B antitoxin." To use in the test, physiological saline is added as the reconstituent.

#### Standard Botulism Type E Antitoxin

A dried product containing "botulism type E antitoxin." To use in the test, physiological saline is added as the reconstituent.

#### Standard Botulism Type F Antitoxin

A dried product containing "botulism type F antitoxin." To use in the test, physiological saline is added as the reconstituent.

#### Standard Antimeasles Serum (Except for Standard Antimeasles serum for PHA test)

A dried product containing "antimeasles antibody" in ampoules. To use in the test, water for injection is added as the reconstituent.

#### Standard Antimeasles Serum for PHA test (for Antimodification)

A liquid or dried product containing labeled IU of "antimeasles antibody" in ampoules.

#### Standard Antimeasles Serum for PHA test (for Modification)

A dried product containing "antimeasles antibody" in ampoules. To use in the test, water for injection is added as the reconstituent.

#### Standard Mamushi (*Agkistrodon halys*) Antivenin

A dried product containing antilethal potency (9.9 U) per mg and antihemorrhagic potency (16.4 U) per mg of "Mamushi antivenins." The National Institute of Infectious Diseases distributes Reference Mamushi (*Agkistrodon halys*) antivenin diluted to 200 U of anti-lethal potency and 330 U of anti-hemorrhage potency per mL for use in laboratory tests.

#### Reference Anti-HBs Human Immunoglobulin

A dried product containing labeled IU of "anti-HBs antibody" per ampoule.

#### Standard Anti-vaccinia Serum

A dried product containing labeled IU of anti-vaccinia antibody per ampoule.

### 1.3 Nucleic Acid

#### Reference Hepatitis B Virus DNA

A liquid product containing hepatitis B virus DNA at a concentration specified on the label.

### 1.4 Others

#### Reference Standard Endotoxin

This product is reference standard endotoxin of the JP.

#### Reference Standard Activated Protein C for Potency Test

A liquid product containing activated protein C. This product must be stored below -65°C and thawed immediately before use in laboratory tests.

## 2. Test Toxins

#### *Clostridium perfringens* Type A Test Toxin

A dried product containing "*C. perfringens* type A toxin" for use in the potency test of a gas gangrene antitoxin (*C. perfringens* type A). One test dose shall kill half of mice aged 23-29 days in approximately 72 hours, when mixed with 0.2 IU of "gas gangrene antitoxin (*C. perfringens* type A)" and injected intravenously 1 hour thereafter.

#### *Clostridium septicum* Test Toxin

A dried product containing "*C. septicum* toxin" for use in the potency test of a gas gangrene antitoxin (*C. septicum*). One test dose shall kill half of mice aged 23-29 days in approximately 72 hours, when mixed with 0.5 IU of "gas gangrene antitoxin (*C. septicum*)" and injected intravenously 1 hour thereafter.

#### *Clostridium oedematiens* Test Toxin

A dried product containing "*C. oedematiens* toxin" for use in the potency test of a gas gangrene antitoxin (*C. oedematiens*). One test dose shall kill half of mice aged 23-29 days in approximately 72 hours, when mixed with 0.02 IU of "gas gangrene antitoxin (*C. oedematiens*)" and injected intramuscularly 1 hour thereafter.

#### Schick Test Toxin (for animal use)

A dried product containing "diphtheria toxin" for use in the detoxification test of a diphtheria toxoid. The test toxin shall contain toxicities of 80 MRD and a combining power of LR/1,000.

#### Diphtheria Test Toxin (for guinea pigs)

A dried product containing "diphtheria toxin" for use in the potency test of a diphtheria antitoxin. One test dose shall kill guinea pigs weighing approximately 230-270 g in about 96 hours, when mixed with 1 IU of "diphtheria antitoxin" and injected subcutaneously 1 hour thereafter.

#### Diphtheria Test Toxin (for rabbit)

A dried product containing "diphtheria toxin" for use in the potency test of a diphtheria antitoxin. One test dose shall produce a redness lesion of 10 mm in diameter in 48 hours, when mixed with 0.01 IU of "diphtheria antitoxin" and injected intracutaneously into rabbits weighing 2.5 to 4.0 kg 1 hour thereafter.

#### Tetanus Test Toxin

A dried product containing "tetanus toxin" for use in the potency test of a tetanus antitoxin. One test dose shall kill mice aged 23-29 days within 96 hours, when mixed with 0.1 IU of "tetanus antitoxin" and injected subcutaneously 1 hour thereafter.

#### Habu (*Trimeresurus flavoviridis*) Test Toxin (lethal)

A dried product containing Habu toxin (Toxin I) prepared from Habu venom for use in the anti-lethal potency test of a Habu antivenin. One test dose shall kill half of mice aged 23-29 days within 48 hours, when mixed with 10 units of "Habu antivenin" and injected intravenously 1 hour thereafter.

*Habu (Trimeresurus flavoviridis) Test Toxin (HR1)*

A dried product containing Habu toxin (HR1) prepared from Habu venom for use in the anti-HR1 potency test of a Habu antivenin. One test dose shall produce a hemorrhagic spot of approximately 10 mm in diameter in the skin of rabbit weighing approximately 2.0-3.0 kg after 24 hours, when mixed with 1 unit of "Habu antitoxin" and injected intracutaneously 1 hour thereafter.

*Habu (Trimeresurus flavoviridis) Test Toxin (HR2)*

A dried product containing Habu toxin (HR2) prepared from Habu venom for use in the anti-HR2 potency test of a Habu antivenin. One test dose shall produce a hemorrhagic spot of approximately 10 mm in diameter in the skin of rabbit weighing approximately 2.0–3.0 kg after 24 hours, when mixed with 1 unit of "Habu antitoxin" and injected intracutaneously 1 hour thereafter.

*Clostridium botulinum Type A Test Toxin*

A liquid product containing "*C. botulinum* type A toxin" for use in the potency test of a botulism type A antitoxin. One test dose shall kill half of mice aged 23-29 days in 72 hours, when mixed with 0.05 IU of "botulism type A antitoxin" and injected intraperitoneally 1 hour thereafter.

*Clostridium botulinum Type B Test Toxin*

There are two kinds of *C. botulinum* type B test toxins for use in the potency test of a botulism type B antitoxin, one being a liquid product containing "*C. botulinum* type B toxin" derived from a proteolytic strain and the other from a nonproteolytic strain. One test dose shall kill half of mice aged 23-29 days in 72 hours, when mixed with 0.05 IU of "botulism type B antitoxin" and injected intraperitoneally 1 hour thereafter.

*Clostridium botulinum Type E Test Toxin*

A liquid product containing "*C. botulinum* type E toxin" for use in the potency test of a botulism type E antitoxin. One test dose shall kill half of mice aged 23-29 days in 72 hours, when mixed with 0.05 IU of "botulism type E antitoxin" and injected intraperitoneally 1 hour thereafter.

*Clostridium botulinum Type F Test Toxin*

A liquid product containing "*C. botulinum* type F toxin" for use in the potency test of a botulism type F antitoxin. One test dose shall kill half of mice aged 23–29 days in 72 hours, when mixed with 0.05 IU of "botulism type F antitoxin" and injected intraperitoneally 1 hour thereafter.

*Mamushi (Agkistrodon halys) Test Toxin (lethal)*

A dried product containing lethal toxin prepared from Mamushi venom for use in the anti-lethal potency test of a Mamushi antivenin. One test dose shall kill half of mice aged 23-29 days within 48 hours, when mixed with 10 units of "Mamushi antitoxin" and injected intravenously 1 hour thereafter.

Mamushi (*Agkistrodon halys*) Test Toxin (hemorrhagic)

A dried product containing hemorrhagic toxin prepared from Mamushi venom for use in the anti-hemorrhagic potency test of a Mamushi antivenin. One test dose shall produce a hemorrhagic spot of approximately 10 mm in diameter in the skin of rabbit weighing approximately 2.0–3.0 kg after 24 hours, when mixed with 1 unit of "Mamushi antitoxin" and injected intracutaneously 1 hour thereafter.

**3. Others**

Opacity Reference Preparation

A liquid product containing 10 International Opacity Units of hard glass particles per mL.

Standard Albumin for Quantitative Determination of Protein

A dried product containing 20 mg of albumin per vial. Using a draught needle, the interior pressure within a vial is brought back to atmospheric pressure and carefully opened. 10 mL of water is accurately added to the vial to make a solution containing 2 mg of albumin per mL.

## C. Reagents, Test Solutions, Etc.

Reagents, test solutions, etc. specified in this section are used to evaluate the quality of biological and blood products.

The designations in parentheses following the names of substances, e.g., (JP), (Standard), (Special Grade) or (Grade I) guarantee that the substance conforms to the specifications for Reagents, Test Solutions for General Tests Processes and Apparatus of JP, the special grade, or grade I of JIS, respectively.

Any substance to which double asterisks, \*\*, are appended shall be of suitable quality for the purposes of the test which uses it.

### A

#### 1mol/L Acetate Buffer Solution

Add nine parts of the sodium acetate test solution (1 mol/L) to one part of the diluted acetic acid and mix. The pH shall be within the range of 5.55 to 5.75.

#### Acetic Acid

Add water to 36 g of glacial acetic acid to make 100 mL (6 mol/L).

#### Acetic Acid, Diluted (Acetic acid, diluted, JP)

#### Acetic Acid, Glacial (Special Grade)

#### Acetylacetone (Special Grade)

#### Acetylacetone Test Solution (JP)

#### Acetone (Special Grade)

#### Activated Partial Thromboplastin Time Reagent (APTT reagent)

This reagent is a phospholipid extracted from rabbit or bovine brain or human placenta, and is mixed with an activator such as ellagic acid\*\*, kaolin\*\*, or celite\*\* for contact activation.

#### Agar (JP)

#### Albumin

Albumin purified from calf serum without deterioration is a light-yellow or light-brown powder, and conforms to the following specifications.

- (1) 10 w/v% albumin solution is a clear liquid and has a pH of 5.0–5.5.
- (2) The content of albumin shall be no less than 97% of total protein upon analysis by gel-electrophoresis.

#### Alkaline Copper Test Solution

Dissolve 0.8 g of sodium hydroxide in water to make 100 mL. Dissolve 4 g of sodium bicarbonate in the solution (Solution A). Mix 1mL of 2 w/v% copper sulfate solution and 1mL of 4 w/v% sodium tartarate solution (Solution B). Mix 50 mL of Solution A and 1mL of Solution B at each use.

#### 0.1 w/v% Aluminium Standard Solution

Measure 895 mg of aluminium chloride and dissolve in water to make 100 mL accurately.

#### Aluminum chloride (Aluminum (III) chloride hexahydrate, Special Grade)

#### Aluminium Hydroxide Test Solution

To 12.5 mL of ammonia solution, add water to make 25 mL (solution a). Dissolve 5.5 g of ammonium sulfate in 150 mL of water at 63°C (solution b). Mix solutions a and b and allow to stand at 58°C (solution c). Dissolve 19.2 g of aluminium ammonium sulfate in 250 mL of water at 58°C (solution d). Mix solutions c and d and keep the mixture standing at 61°C. Shake thoroughly for 10 minutes while maintaining the temperature at higher than 58°C. Centrifuge the mixture at  $800 \times g$  for 5 minutes. Discard the supernatant. Add 0.055 mL of ammonia solution to the precipitate and then add water to make a final volume of 375 mL. Centrifuge it again. After discarding the supernatant, Add 0.11 mL of ammonia solution to the precipitate and then add water to make 375 mL. Centrifuge it. Dispense the final precipitate in water to make 175 mL. Store the suspension in the dark and use within 6 weeks of preparation.

Aminoacetic Acid (Glycine, Special Grade)

0.30 mol/L Aminoacetic Acid Test Solution

Dissolve 22.5 g of aminoacetic acid in water to make 1000 mL.

1-Amino-2-Naphthol-4-Sulfonic Acid (Special Grade)

1-Amino-2-Naphthol-4-Sulfonic Acid Test Solution

Dissolve 0.5 g of 1-amino-2-naphthol-4-sulfonic acid in approximately 100 mL of 15 w/v% sodium bisulfite solution in a 200 mL volumetric flask. Warm gently to dissolve thoroughly, if necessary. Add 5 mL of 20 w/v% sodium sulfite solution and agitate the solution. Add a suitable volume of 15 w/v% sodium sulfite solution to make 200 mL finally. The solution can be used for up to 2 weeks after preparation when kept cooled in a dark amber bottle.

Ammonia Solution (Special Grade: 28%)

9mol/L Ammonia Solution

Add water to 60 mL of ammonia solution to make 100mL.

Ammonium Acetate (Special Grade)

Ammonium Aluminium Sulfate [K8087:1993, Aluminium ammonium sulfate dodecahydrate (Ammonium alum), Special Grade]

Ammonium Ferric Sulfate Test Solution [Ammonium iron (III) sulfate test solution, JP]

Ammonium Molybdate [Hexammonium Heptamolybdate Tetrahydrate. Special Grade]

Ammonium Persulfate (Ammonium peroxodisulfate, Special Grade)

Ammonium Sulfate (Special Grade)

0.1 mol/L Ammonium Thiocyanate (JP, Standard solution for volumetric analysis)

Anthrone (Special Grade)

Anthrone Sulfuric Acid Test Solution

Add 66 mL of sulfuric acid to 34 mL of water and then cool the solution. Add 50 mg of anthrone to the solution and dissolve it. Then, add 1g of thiourea to the solution and dissolve it. The test solution is stored at 2–10°C in a dark amber bottle and can be used for up to 2 weeks after preparation.

## **B**

Barium hydroxide [Barium hydroxide octahydrate, Special Grade]

Seal containers tightly.

Barium sulfate [K8991:1961, Special Grade]

Benzoic Acid (Special Grade)

Borate Buffer Solution, Sørensen's (pH 7.45)

Add 35 mL of 0.2 mol/L hydrochloric acid test solution to 65 mL of 0.20 mol/L sodium borate test solution.

0.05 mol/L Borate Buffered Sodium Chloride Solution containing 0.02 w/v% Gelatin (pH 7.45)

Mix 2 mL of 10 w/v% gelatin solution, 250 mL of borate buffer solution, Sørensen's (pH 7.45), and 10 mL of 1 w/v% thimerosal solution. Dilute the mixture with physiological saline to make 1,000 mL. Sterilize the solution.

Bouillon solution

Add 10 g of peptone\*\* and 5 g of sodium chloride to 1000 mL of broth. \*\* Boil for about 30 minutes. After cooling, filtrate and sterilize and adjust the pH to approximately 7.2.

Bovine Heart Extract

Add twice the volume of water to chopped bovine heart muscle. Stir the mixture and allow to stand at 2–5°C overnight. Extracted in a boiling water bath for 1 hour and then filter. Add peptone\*\* and sodium chloride to the filtrate to be 1.0 w/v% and 0.5 w/v%, respectively.

Bromine (Special Grade)

Bromine Test Solution (JP)

Bromthymolblue (Special Grade)

Bromthymolblue Test Solution (JP)

## C

Calcium Chloride anhydrate (Calcium chloride, Special Grade)

Calcium chloride (Calcium chloride dihydrate, Special Grade)

0.05 mol/L Calcium Chloride Test Solution

Dissolve 7.38 g of calcium chloride in water to make 1000 mL.

0.025 mol/L Calcium Chloride Test Solution

Dissolve 3.68 g of calcium chloride in water to make 1000 mL.

Calcium Chloride Solution containing Thrombin

Dissolve 60 units of thrombin in 3.5 mL of 0.05 mol/L calcium chloride test solution.

Carbon Tetrachloride (Special Grade)

Chloroacetic Acid (Special Grade)

Chloroform (Special Grade)

Citric Acid (Citric Acid monohydrate, Special Grade)

Copper Sulfate (Copper Sulfate (II) pentahydrate, Special Grade)

Cyanmethemoglobin Standard Solution

The solution conforms to the specifications of the International Committee for Standardization in Hematology and contains 55–85 mg of cyanmethemoglobin per 100 mL.

L-Cystine [L-(-)-Cystine, Special Grade]

## D

### 2,7-Diaminofluorene Dihydrochloride ( $\text{H}_2\text{NC}_6\text{H}_3\text{CH}_2\text{C}_6\text{H}_3\text{NH}_2 \cdot 2\text{HCl}$ MW = 269.17)

Description: 2,7-Diaminofluorene dihydrochloride is a white powder, readily soluble in water, and almost insoluble in alkaline buffer. When dimethylsulfoxide is added, it becomes soluble even in alkaline buffer.

Storage: Preserve in a dry and cold place.

### *o*-Dianisidine $\text{CH}_3\text{OC}_6\text{H}_3(\text{NH}_2)\text{C}_6\text{H}_3(\text{NH}_2)\text{OCH}_3$ , MW=244.29

Description: *o*-Dianisidine occurs as white or purple crystals or a white crystalline powder. It is soluble in ethanol, ether or acetone, and insoluble in water.

Melting point: 135–139°C

### 2,7-diamino Dienes' Staining Solution

|                  |        |
|------------------|--------|
| Methyleneblue    | 2.5 g  |
| Azur II **       | 1.25 g |
| Maltose          | 10.0 g |
| Benzoic acid     | 0.25 g |
| Sodium carbonate | 0.25 g |

Dissolve the above ingredients in water to make 100 mL.

### 5, 5-Diethylbarbitic Acid (Barbital, JP)

### *p*-Dimethylamidobenzaldehyde (Special Grade)

### Dithizone [Dithizone (1,5-diphenylthiocarbazone), Special Grade]

### Dithizone Test Solution

Dissolve 2.0 mg of dithizone in carbon tetrachloride to make 100 mL.

### Distilled Water (Water for injection, JP, prepared by distillation.)

### Dulbecco Phosphate-buffered Sodium Chloride Solution (pH 7.4)

Dissolve 8.0 g of sodium chloride, 0.2 g of potassium chloride, 1.15 g of sodium monohydrogen phosphate anhydrous, and 0.2 g of sodium dihydrogen phosphate in water to make 1000 mL.

### Dulbecco Phosphate-buffered Saline Containing 0.05 w/v% Sodium Azide (pH 7.4)

Dissolve 0.05 g of sodium azide in 100 mL of Dulbecco phosphate-buffered saline (pH 7.4).

## E

### Ethanol (Ethanol (95), Special Grade)

### Ethanol, Diluted (Ethanol, diluted, JP)

### Ether (Diethylether, Special Grade)

### Ether, Anhydrous [Ethyl ether, Special Grade, The water content is not more than 0.01%]

## F

### Factor VIII-depleted Human Plasma

This product is human plasma from which factor VIII has been selectively depleted using the immunological absorption method.

#### Factor IX-depleted Human Plasma

This product is human plasma from which factor IX has been selectively depleted using the immunological absorption method.

#### Ferrous Sulfate (Iron (II) sulfate heptahydrate, Special Grade)

##### Ferrous Sulfate Test Solution

Dissolve 200 g of ferrous sulfate in water with warming, if necessary. Adjust the volume to 500 mL with water. Add 5.0 mL of sulfuric acid to the solution.

#### Fibrinogen

Fibrinogen purified from human plasma without deterioration is a white or light-yellow powder, and conforms to the following specifications.

- (1) Its 2 w/v% solution is clear and has a pH of 6.0–7.3.
- (2) Content of clottable protein shall be more than 80% of total protein, when the amounts of total protein and clottable protein are measured by the Kjeldahl method. The clot is obtained by treating the test sample with sufficient amounts of thrombin and calcium in the range of pH 6.6–7.4 at 20–30°C. It is thoroughly washed with a suitable solution, and used for the determination of clottable protein.
- (3) Add 0.1 mL of physiological saline containing albumin and 0.1 mL of calcium chloride solution containing thrombin to 0.1 mL of a 1% solution of the test sample. Incubate the mixture for 1 hour at 37°C to produce the clot. This clot shall be dissolved within 60 seconds upon the addition of 3 mL of 1 w/v% chloroacetic acid.

#### Fibrinogen Solution

Dissolve 50 mg of fibrinogen in 5 mL of physiological saline.

#### Folin's Test Solution (JP)

#### Four-units Measles Antigen Solution

Take an appropriate amount of measles HA-antigen according to the following method of HA-antigen measurement. Dilute the measles HA-antigen with 0.067 mol/L phosphate-buffered sodium chloride solution containing 0.1 w/v% albumin and 0.01 w/v% gelatin to prepare a solution containing four units per 25 µL of the measles HA-antigen.

Measurement of measles HA-antigen content:

Prepare and take a series of two-fold dilutions of measles HA-antigen in a test tube. Add 25 µL of 0.067 mol/L phosphate-buffered sodium chloride solution containing 0.1 w/v% albumin and 0.01 w/v% gelatin (pH 7.2) and then 50 µL of 0.5 vol% of green monkey red blood cell suspension to the tube and gently mix. Keep the tubes standing at 37°C for 2 hours and observe aggregation macroscopically. The highest dilution concentration that causes aggregation shall be taken as the antigen dilution titer and expressed as “the number of dilutions” with unit.

## G

#### Glucose (JP)

##### 0.01 w/v% Glucose Standard Solution

Measure 100 mg of glucose accurately. Dissolve it in water to make 100 mL accurately, then

dilute the solution accurately 10-fold with water.

## H

### Hemoglobin Solution

Rinse human red blood cells with physiological saline, and lysis them with water. After centrifugation to remove stroma, obtain clear hemoglobin solution. Determine the concentration of hemoglobin in the solution by the cyanmethohemoglobin method. Adjust the hemoglobin concentration to a constant value. After the adjustment, the correct hemoglobin content shall be determined again.

### Heparin sodium [JP]

### Hexamethylenetetramine (Special Grade)

### Histamine Dihydrochloride (Special Grade)

White or slightly yellow crystalline powder. It is soluble in water. Its content is 97.0% or more.

### Human Serum Albumin

Human Serum Albumin is a pale-yellow or yellow-brown powder that is prepared from human plasma using a method shown not to cause the deterioration of albumin and other plasma proteins. When the cellulose acetate membrane electrophoretic test given in General Tests is applied, no less than 96% of the total proteins shall have the mobility of serum albumin.

### Hydrochloric Acid (Special Grade)

1 mol/L Hydrochloric Acid Test Solution [Hydrochloric Acid Test Solution, 1 mol/L, JP]

0.5 mol/L Hydrochloric Acid Test Solution [Hydrochloric Acid Test Solution, 0.5 mol/L, JP]

0.2 mol/L Hydrochloric Acid Test Solution [Hydrochloric Acid Test Solution, 0.2 mol/L, JP]

0.1 mol/L Hydrochloric Acid Test Solution [Hydrochloric Acid Test Solution, 0.1 mol/L, JP]

Hydrogen Peroxide Test Solution (JP)

## I

### Indol Test Solution

Dissolve 1 g of p-dimethylaminobenzaldehyde in 95mL of ethanol, and add 20 mL of hydrochloric acid.

### Isotonic Sodium Chloride Solution (Physiological saline, JP)

### Isotonic Sodium Chloride Solution containing Albumin

Dissolve 0.4 g of albumin in physiological saline to make 100 mL.

### Physiological saline containing 0.015 M Sodium Citrate

Dissolve 0.44 g of sodium citrate in physiological saline to make 100 mL.

### Physiological saline containing 0.01 w/v% Thimerosal

Dilute 10 mL of 1 w/v% thimerosal solution with physiological saline to make 1000 mL.

### <sup>125</sup>I-labeled HBs antigen solution

A solution of HBs antigenic protein\*\* labeled with a radioisotope <sup>125</sup>I\*\*.

## **K**

### Kaolin test solution

Suspend 25 g of kaolin\*\* in a suitable volume of 0.067 mol/L phosphate-buffered sodium chloride solution (pH 7.2). Make 100 mL with the same buffer solution.

## **L**

### Lactose [Lactose monohydrate, Special Grade]

#### 0.01 w/v% Lactose Standard Solution

Dissolve 100 mg of lactose in water to make 100 mL. Dilute one volume of this solution 10 times with water accurately.

### Lead acetate [Lead (II) acetate trihydrate, Special Grade]

### Lead acetate paper [JP]

## **M**

### Magnesium Sulfate [Magnesium sulfate heptahydrate, Special Grade]

### Maltose [K8883:1992, Maltose monohydrate (maltose), Special Grade]

### Methyleneblue (Special Grade)

#### 5 w/v% Mucine Solution

Suspend 100 g of gastric mucine\*\* with 2000 mL of water and warm the suspension at 56–58°C with constant shaking to make a viscous solution. Filter the solution through several layers of gauze and sterilize. Store at 2–5°C under avoidance of light. The solution is available for use for 6 months. Immediately before use, warm the solution to 37°C and adjust the pH to 7.2–7.4. Unused warmed solution is discarded.

## **N**

### Nitric acid [Special Grade, Density: approximately 1.42 g/mL]

### Nitric acid, Fuming [Special Grade]

#### Nitric acid•Acetic Acid Test Solution

Dilute 3 mL of nitric acid and 46 mL of glacial acetic acid with water to make 1000 mL.

### *p*-Nitroaniline [4-Nitrobenzenamine, Special Grade]

#### *p*-Nitroaniline - Sodium Nitrite Test Solution

Dissolve 1.50 g of *p*-nitroaniline in 40 mL of hydrochloric acid. Add water to make 500 mL.

Warm the solution in a water bath if necessary. Add 0.75 mL of sodium nitrite test solution to 25 mL of the solution. The mixed solution shall be newly prepared at each use.

### Neutral Red (K8729:1992, Special Grade)

## **P**

### Paraffin, Liquid (Special Grade)

### Peptone of Casein Origin

The substance is a grayish yellow powder with a characteristic odor but without a putrid smell.

It is soluble in water, but insoluble in ethanol or ether.

Test for the degree of digestion: Dissolve 1 g of the substance in 10 mL of water to make the test material, which is subjected to the tests given below.

(1) When 1 mL of the test material is overlaid with 0.5 mL of a solution prepared by mixing 10 mL of diluted ethanol and 1 mL of glacial acetic acid, no ring zone or precipitate shall be observed at the marginal surface. When the tube is shaken to mix the contents, no turbidity shall occur.

(2) When 1 mL of the test material is combined with 4 mL of saturated zinc sulfate solution, a small amount of precipitate (proteose) shall be formed.

(3) When 1 mL of the filtrate from the above test (2) sample is combined with 3 mL of water and 0.2 mL of the bromine test solution, a red-purple coloring shall be observed.

Phenol (Special Grade)

0.5 w/v% Phenol Standard Solution

Measure 5 g of phenol accurately. Dissolve in water to make 1000 mL accurately.

Phenolphthalein (Special Grade)

Phenolphthalein Test Solution (JP)

Phenol red (Special Grade)

Phenol red Test Solution (JP)

0.1 mol/L M Phosphate Buffer Solution containing 0.1 w/v% Sodium Dodecyl Sulfate (pH 7.2)

Dissolve 15.6 g of sodium dihydrogen phosphate, 1.0 g of sodium dodecyl sulfate, and 0.5 g of sodium azide in a suitable volume of water. Adjust the pH of the solution to 7.2 with an appropriate amount of sodium hydroxide. Add water to the solution to make 100 mL.

0.0067 mol/L Phosphate-buffered sodium chloride solution (pH 7.6)

Dissolve 80.0 g of sodium chloride, 2.0 g of potassium chloride, 11.5 g of sodium monohydrogen phosphate, and 2.0 g of sodium dihydrogen phosphate in water to make 1000 mL and sterilize the solution.

0.010 mol/L Phosphate-buffered sodium chloride solution (pH 7.0–7.2)

Dissolve 83.0 g of sodium chloride, 25.1 g of sodium monohydrogen phosphate, and 4.08 g of potassium dihydrogen phosphate in water to make 1000 mL and sterilize the solution.

0.013 mol/L Phosphate-buffered sodium chloride solution (pH 7.0)

Dissolve 85.0 g of sodium chloride, 11.56 g of sodium monohydrogen phosphate anhydrous, and 7.06 g of potassium dihydrogen phosphate in water to make 1000 mL and sterilize the solution.

0.017 mol/L Phosphate-buffered sodium chloride solution (pH 7.0)

Dissolve 85.0 g of sodium chloride, 14.45 g of sodium monohydrogen phosphate anhydrous, and 8.83 g of potassium dihydrogen phosphate in water to make 1000 mL and sterilize the solution.

0.067 mol/L Phosphate-buffered sodium chloride solution (pH 7.2)

Dissolve 85.0 g of sodium chloride, 16.71 g of sodium monohydrogen phosphate, and 2.72 g of potassium dihydrogen phosphate in water to make 1000 mL and sterilize the solution.

0.067 mol/L Phosphate-buffered sodium chloride solution (pH 7.2) containing 0.1 w/v% Albumin

and 0.01 w/v% Gelatin

Dissolve 1.0 g of albumin and 0.1 g of gelatin\*\* in a suitable volume of 0.067 mol/L phosphate-buffered sodium chloride solution (pH 7.2) with heating. Add 0.067 mol/L phosphate-buffered sodium chloride solution (pH 7.2) to make 1000 mL.

0.017 mol/L Phosphate-buffered Sodium Chloride Solution containing 0.2 w/v% Gelatin (pH 7.0)

Dissolve 0.2 g of gelatin \*\* in a suitable volume of 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) with heating. Adjust the volume to 100 mL with the same buffer solution and sterilize the solution.

0.017 mol/L Phosphate-buffered Sodium Chloride Solution containing 0.02 w/v% gelatin (pH 7.0)

Dissolve 0.02 g of gelatin \*\* in a suitable volume of 0.017 mol/L phosphate-buffered sodium chloride solution (pH 7.0) with heating. Adjust the volume to 100 mL with the same buffer solution and sterilize the solution.

0.0067 mol/L Phosphate-buffered Sodium Chloride Solution containing 0.2 w/v% gelatin (pH 7.6)

Dissolve 0.2 g of gelatin \*\* in a suitable volume of 0.0067 mol/L phosphate-buffered sodium chloride solution (pH 7.6) with heating. Adjust the volume to 100 mL with the same buffer solution and sterilize the solution.

Phosphate Standard Solution (0.11 mg of potassium dihydrogen phosphate per mL of water)

Dry potassium dihydrogen phosphate at 100-110°C for 1 hour and cool it in a desiccator. Measure 0.11 g of the potassium dihydrogen phosphate accurately and dissolve it in water to make 1000 mL.

Phosphorus Pentoxide [Phosphorus (V) oxide, Special Grade]

Physiological Saline

See "Isotonic Sodium Chloride Solution".

Polyacrylamide Gel

Dissolve 19 g of acrylamide\*\*, 1g of methylenebisacrylamide\*\*, and 9.12 g of tris (hydroxymethyl) aminomethane in an appropriate amount of water and then mix with 12 mL of 1 mol/L hydrochloric acid. Add water to make 200 mL (a).

Dilute 1 mL of tetramethylenediamine\*\* with water to make 100 mL(b).

Dissolve 120 mg of ammonium persulfate in 100 mL of water (c). The solution is freshly prepared before use.

Mix (a), (b) and (c) in a ratio of 2:1:1.

Polyacrylamide Gel containing Sodium Dodecyl Sulfate

Dissolve 6.24 g of sodium dihydrogen phosphate, 2 g of sodium dodecyl sulfate\*\* and 0.4 mL of tetramethylenediamine\*\* in 80 mL of water.

Adjust the pH of the solution to 7.2 with 2 mol/L sodium hydroxide test solution and add water to make 100 mL (a).

Dissolve 20 g of acrylamide\*\* and 0.7 g of methylenebisacrylamide in water to make 100 mL (b).

Dissolve 60 mg of ammonium persulfate in 10 mL of water (c). The solution is freshly prepared before use.

Mix the solutions (a), (b), (c), and water in a ratio of 3:3.5:1:4.5.

#### Ponceau 3R staining solution

Dissolve 0.4–0.8 g of ponceau 3R and 6.0 g of trichloroacetic acid in water to make 100 mL.

#### Potassium Standard Solution (JP)

Potassium Bromide (Special Grade)

Potassium Chloride (Special Grade)

Potassium Cyanide (Special Grade)

Potassium Dihydrogen Phosphate [Potassium dihydrogen phosphate, Special Grade]

Potassium Ferricyanide (Potassium hexacyanoferrate(III), Special Grade)

Potassium Iodine (Special Grade)

Potassium Monohydrogen Phosphate (Dipotassium hydrogen phosphate, Special Grade)

Potassium Permanganate (Special Grade)

Potassium Permanganate Test Solution (JP)

Potassium Sodium Tartrate (Potassium Sodium Tartrate Tetrahydrate (Rochelle Salt or Seignette's Salt, Special Grade)

## R

#### 3 vol% Red Blood Cell (O phenotype in Rho-positive site) Suspension

Wash one volume of red blood cells (O phenotype in Rho-positive site) three times with physiological saline. Suspend in approximately 30 volumes of physiological saline.

## S

#### Sensitized sheep red blood cell suspension

Adjust the concentration of sheep red blood cells to  $1 \times 10^9$  cells/mL in an appropriate buffer.

Incubate the cell suspension with suitably diluted hemolysin\*\* (anti-sheep red blood cell antibody) in the same buffer at 37°C for 20 minutes.

#### Silica Gel (JP)

0.01 mol/L Silver nitrate solution (JP, standard silver solution for atomic absorption spectrophotometry)

Sodium Acetate [Sodium acetate trihydrate, Special Grade]

Sodium acetate test solution [JP]

Sodium Azide (Special Grade)

Sodium 5,5-diethylbarbiturate (Barbital sodium, JP)

Sodium diethylbarbiturate buffer solution (pH 8.6)

Dissolve appropriate amounts of Sodium 5,5-diethylbarbitate and 5,5-diethylbarbituric acid in the ratio of 28:5 by weight in a suitable volume of water. Adjust the volume to make a suitable ionic strength with water.

0.1 mol/L sodium diethylbarbiturate Test Solution

Dissolve 20.7 g of sodium diethylbarbitate in water to make 1000 mL.

Sodium Bicarbonate (Sodium Bicarbonate, Special Grade)

Sodium Bisulfite (Special Grade)

Sodium Borate [Sodium tetraborate decahydrate, Special Grade]

0.20 mol/L Sodium Borate Solution

Measure 76.29 g of sodium borate. Dissolve in water to make 1000 mL accurately.

Sodium Carbonate (Sodium Carbonate Decahydrate, Special Grade)

Sodium Carbonate Anhydrous (Sodium Carbonate, Special Grade)

Sodium Carbonate Test Solution (JP)

Sodium Chloride (Special Grade)

Sodium Chloride (for Culture Media) (JP)

0.6 w/v% Sodium Chloride Solution containing 1 w/v% Peptone of Casein Origin

Dissolve 1 g of peptone of casein origin and 0.6 g of sodium chloride in water to make 100 mL.

Sterilize the solution by autoclaving at 121°C for 20 minutes.

Sodium Citrate (Trisodium citrate dihydrate, Special Grade)

Sodium Diethylbarbiturate-Hydrochloric Acid Buffer Solution containing Calcium Chloride

Dissolve 500 mg of calcium chloride in 554 mL of 0.10 mol/L sodium diethylbarbiturate test solution. Add 446 mL of 0.1 mol/L hydrochloric acid test solution to this solution. The pH of the solution indicates the value of 7.2–7.4.

Sodium dihydrogen phosphate [Sodium dihydrogen phosphate dihydrate, Special Grade]

Sodium Hydroxide [Special Grade]

2.5 mol/L Sodium Hydroxide Test Solution

Dissolve 10.7 g of sodium hydroxide in water to make 100 mL.

2 mol/L Sodium Hydroxide Test Solution

Dissolve 8.6 g of sodium hydroxide in water to make 100 mL.

1 mol/L Sodium Hydroxide Test Solution

Dissolve 4.3 g of sodium hydroxide in water to make 100 mL.

0.1 mol/L sodium hydroxide [JP, standard solution for volumetric analysis]

Sodium Monohydrogen Phosphate [Disodium hydrogen phosphate dodecahydrate, Special Grade]

Sodium Monohydrogen Phosphate Anhydrous [disodium hydrogen phosphate, Special Grade]

Sodium Nitrite (Special Grade)

Sodium Nitrite Test Solution (JP)

Sodium Oxalate [Sodium Oxalate, Special Grade]

0.05 mol/L Sodium Oxalate Test Solution

Dissolve 6.7 g of sodium oxalate in water to make 1000 mL.

Sodium tartrate [(+)-Sodium Tartrate dihydrate, Special Grade]

0.1 w/v% Sodium Standard Solution

Measure 2.542 g of sodium chloride. Dissolve in water to make 1000 mL accurately.

Sodium Sulfite anhydrate (Sodium Sulfite, Special Grade)

Sodium Sulfide Test Solution (JP)

Sodium tartrate ((+)-Sodium Tartrate dihydrate, Special Grade)

Sodium Thioglycollate (K8631:1961, Special Grade)

Preserve in containers, protected from light in a dark, cold place.

0.04 w/v% Standard Solution for Formaldehyde Analysis

Dissolve 311 mg of hexamethylenetetramine in water to make 1000 mL. The solution corresponds to 400 µg/ mL of formaldehyde.

Stilbazo

Stilbene-4, 4'-bis [(1-azo)-3, 4-dihydroxybenzene]-2,2'-disulfonic acid diammonium salt

- (1) The substance is a black-brown powder.
- (2) When dissolved in water, the color of the solution is yellow in the pH range of 3–7, orange at pH 9 and red at pH 11. When 50 mg of the substance is dissolved in 100 mL of water, no insoluble portion shall remain.
- (3) When 0.5 g of the substance is rendered to ash with 1mL of sulfuric acid, the ashes shall not exceed 10 mg in a constant weight.
- (4) The light absorbance of 0.02 w/v% solution at the wave length of 410 nm shall be 0.7 or more.
- (5) A 5-mL portion of 0.05 w/v% solution is combined with 10 mL of a 0.0001 M aluminium chloride solution and 10 mL of 1mol/L acetate buffer solution and diluted with water to 100 mL. The absorbance of the dilution at the wave length of 505 nm and the light path of 10mm shall be higher by at least 0.42 than that of the dilution containing stilbazo alone.

Stilbazo Test Solution

Grind approximately 50 mg of stilbazo in a mortar. Add water to make 100 mL and filtrate the mixture. Add 10 mL of 1mol/L acetate buffer solution and 14 mL of water to 1 mL of the filtrate and stand at approximately 25°C for 20 minutes. The absorbance of the mixture at 420 nm and light path of 10 mm shall be 0.85 or more. When the solution is stored in the dark at 2–5°C, it can be used for up to 2 weeks after preparation.

Sucrose, Purified (JP)

20% Sucrose Solution, for Fractionation

Dissolve 20 g of white soft sugar in 80 g of physiological saline containing 0.015 mol/L sodium citrate.

50% Sucrose Solution, for Fractionation

Dissolve 50 g of white soft sugar in 50 g of physiological saline containing 0.015 mol/L sodium citrate.

Sulfamic acid (amidosulfuric acid (standard reagent), standard substance for volumetric analysis)

Sulfuric acid (Special Grade)

0.5 mol/L Sulfuric acid test solution

Add slowly 28 mL of sulfuric acid to 500 mL of water with stirring, and then cool the solution. Adjust the volume to 1000 mL with water.

Sulfuric acid, dilute [Sulfuric acid, dilute, JP]

Sulfuric Acid, Fuming (Special Grade)

## T

### Thimerosal $C_9H_9HgNaO_2S$

Description: Thimerosal is white or light-yellow crystalline powder with a slight, characteristic odor. In solution (1.0 g in 100 mL of water) it has a pH of 6.0–7.0.

Purity test:

- (1) Clarity and color of solution -- Clear, colorless solution (1.0 g in 10 mL of water)
- (2) Ether-soluble substances -- Approximately 0.5 g of the pulverized sample is precisely weighed and added to 20 mL of anhydrous ether in a 50 mL glass-stoppered Erlenmeyer flask. The mixture is shaken for 10 minutes under tightly stoppered conditions and filtrated into a pre-weighed beaker through a filter paper pre-washed with ether. Residues on the filter paper are washed with 5 mL of anhydrous ether. The combined filtrates are evaporated on a steam bath and dried for 24 hours in a desiccator under reduced pressure with silica gel. The amount of extracted substance is not more than 0.60% of the weight of the sample.
- (3) Foreign water-soluble mercury salts -- A 0.10 g quantity of the sample is dissolved in 10 mL of water. The addition of 3 drops of acetic acid to 5 mL of the solution causes the precipitation of a white material, and the further addition of 1 drop of the sodium sulfide test solution does not turn the solution to black upon standing for 10 minutes.
- (4) Readily carbonizable substances -- A 0.20 g quantity of the sample is weighed and tested according to General Tests, Processes and Apparatus in the JP. The test is carried out at 20°C. The color of the sample solution is not deeper than that of Matching Fluid J.

Loss on Drying: Not more than 0.5% of the weight of the sample. A precisely weighed sample of approximately 1 g is dried for 5 hours in a desiccator under reduced pressure with silica gel.

Content: Not less than 98%

Assay: Approximately 0.3 g of the dried sample is precisely weighed and put into a 300 mL Kjeldahl flask. To the sample 10 mL of sulfuric acid and 4 mL of fuming nitric acid are successively added. The mixture is heated at first gently and then gradually ignited on a sand bath until the contents become almost colorless and white fumes are evolved. After cooling, the contents are quantitatively transferred into a beaker with 100 mL of water and heated in a steam bath for 15 minutes with occasional shaking. To the solution, 0.5 g of urea is added with shaking, and then the potassium permanganate test solution is added dropwise until a faint red color appears. To the cooled solution, the hydrogen peroxide test solution is added dropwise until the red color of the solution disappears. This solution is titrated with 0.1 mol/L ammonium thiocyanate solution (Indicator: 2 mL of ammonium ferric sulfate test solution).

1 mL of 0.1 mol/L ammonium thiocyanate solution = 20.240 mg  $C_9H_9HgNaO_2S$

Storage: Preserve in tightly sealed, light-shielded containers.

### 0.02 w/v% Thimerosal Standard Solution

Measure 20 mg of thimerosal accurately. Dissolve in water to make 100 mL accurately.

Trichloroacetic acid (Special Grade)

Tris(hydroxymethyl)aminomethane (2-amino-2-hydroxymethyl-1,3-propanediol, Special Grade)

Thiourea (Special Grade)

Thrombin (JP)

Thromboplastin Solution

The solution is prepared by either of the following two procedures.

Procedure (1): Triturate fresh bovine or rabbit brain from which the meninges have been removed as completely as possible.

Add five volumes or more of acetone at once to the triturated brain, and centrifuge. Wash the precipitate four times with acetone, and dry rapidly at room temperature. Soak 200 mg of the dried powder in 5 mL of water for injection or physiological saline to extract thromboplastin. Centrifuge at  $1,000 \times g$  for 5 minutes. After centrifugation, use the supernatant formed.

Procedure (2): Cut peripheral portions of fresh bovine lung free from bronchi into approximately 5 cm cubes. Wash with water to remove blood, and mince through holes of 3–5 mm diameter. Soak the minced lung in an equal volume of physiological saline with stirring occasionally at approximately 5°C for 48–72 hours, and then centrifuge it. Store the supernatant at 2–10°C. Immediately before use, dilute the supernatant 5- to 10-fold with water for injection or with physiological saline.

Assay: The prothrombin time of the solution shall not exceed 15 seconds against normal human plasma. The prothrombin time represents the time needed for the formation of a fibrin clot from the moment when one volume of human plasma (centrifugal supernatant of the mixture of nine volumes of blood and one volume of 0.05 mol/L sodium oxalate solution or 0.033 mol/L sodium citrate solution as anticoagulant) is mixed with one volume of the solution, prepared as above, and then with one volume of a 0.025 mol/L calcium chloride test solution.

Trichloroacetic Acid (Special Grade)

Tris(hydroxymethyl)aminomethane(2-Amino-2-hydroxymethyl-1,3-propanediol, Special Grade)

## U

Urea (Special Grade)

8 mol/L Urea Solution containing 1 w/v% Sodium Dodecyl Sulfate

Dissolve 0.09 g of sodium dodecyl sulfate\*\*, 4.8 g of urea, and 0.0185 g of iodoacetamide \*\* in 0.1 mol/L phosphate buffer solution containing 0.1 w/v% of sodium dodecyl sulfate (pH 7.2) to make 10 mL.

## V

van Kampen Reagent Solution (pH 7.2)

Dissolve 200 mg of potassium ferricyanide, 50 mg of potassium cyanide, an appropriate amount of potassium dihydrogen phosphate, and an appropriate amount of detergent\*\* in water to make 1000 mL.

**W**

Water for Bacterial Endotoxins Test (JP)

Water for Injection (JP)

**Y**

Yeast Extract (JP)

**Z**

Zinc sulfate [Zinc sulfate heptahydrate, Special Grade]

## D. Buffered Solutions and Culture Media

### General rules

1. The specified pH value shall be that after sterilization.
2. To "sterilize" usually means to sterilize by autoclaving at 121°C for 15 minutes.
3. Culture media of the proper quality according to the purpose of the test can be used.
4. "Make to plate" means the following procedure: melt the medium by heating, cool to a suitable temperature, pour approximately a 20-mL portion into a Petri dish of approximately 9cm in diameter, and allow to solidify.

### EC Medium

A dried commercial product of appropriate quality is dissolved according to the direction, dispensed usually in 10 mL volumes and sterilized. The pH shall be 6.8–7.0.

### Cooked Meat Medium

Meat peptone 8.0 g

Cooked meat 4.0 g

The above ingredients are combined with water to make 1,000 mL. Heating is allowed, if necessary. The pH shall be 7.2–7.6. Immediately before inoculation, a sufficient amount of sterilized 50 w/v% glucose solution is added to 1 w/v%. Any above-named dried medium of a suitable quality treated according to the direction may be used.

### Blood Agar Basic Medium

Meat extract 10 g

Pepton 10 g

Sodium Chloride 5 g

Agar 15 g

Above ingredients are combined with water to make 1,000 mL. The pH shall be 7.4–7.6.

### Blood Agar Medium

Sterilize the above blood agar basic medium. After cooling to 50°C, sheep or rabbit blood is added aseptically to a final concentration of 5%.

### Colombia Agar Medium

Any dried medium of suitable quality is dissolved according to the direction and sterilized. The pH shall be 7.1–7.5.

### Thioglycollate Agar Medium

Agar 15.0 g

L-cystine 0.5 g

Sodium Chloride 2.5 g

Peptone made from casein 15.0 g

Yeast extract 5.0 g

Glucose 5.0 g

Sodium Thioglycollate 0.5 g

Water 1000 mL

Adjust pH 7.0–7.2

Any medium of suitable quality can be used according to the direction in place of the above ingredients listed. Sodium Thioglycollate can be omitted except when the medium is used for testing a sample containing thimerosal as a preservative.

#### Brain Heart Infusion Broth

Any dried medium of suitable quality is dissolved according to the direction and sterilized. The pH shall be 7.2–7.6.

#### Pepton Sodium Chloride Buffer

Potassium Dihydrogen Phosphate 3.56 g

Sodium Dibasic Phosphate 18.23 g

Sodium Chloride 4.30 g

Pepton 1.0 g

Water 1000 mL

Mixed all the above-listed ingredients and sterilize at 121°C for 15 to 20 minutes. The pH shall be 6.9–7.1. Polysorbate 20 or 80 (0.1–1.0 w/v%) can be added.

#### Media for Mycoplasma Test

##### 1. Broth Medium for Mycoplasma Testing I

(Basic Medium)

Beef heart infusion broth (pH 7.8–8.0) 75 mL

Glucose 0.3 g

Phenol red solution (0.5w/v%) 0.5 mL

(Additive)

Equine serum 15 mL

25% fresh yeast extract (pH 7.3–7.5) 10 mL

Penicillin G potassium 50,000 units

The pH shall be 7.6–7.8.

##### 2. Broth Medium for Mycoplasma Testing II

(Basic Medium)

Beef heart infusion broth (pH 7.8–8.0) 75 mL

Arginine hydrochloride 0.3 g

Phenol red solution (0.5w/v%) 0.5 mL

(Additive)

Equine serum 15 mL

25% fresh yeast extract (pH 7.3–7.5) 10 mL

Penicillin G potassium 50,000 units

The pH shall be 7.0–7.2.

3. Agar Plate Medium for Mycoplasma Testing  
(Basic Medium)

Beef heart infusion broth (pH 7.8–8.0) 75 mL

Agar 1.2 g

(Additive)

Equine serum 15 mL

25% fresh yeast extract (pH 7.3–7.5) 10 mL

Penicillin G potassium 50,000 units

Both beef heart infusion broth and fresh yeast extract can be replaced by any product of suitable quality. Equine serum shall be used after heat-treatment at 56°C for 30 minutes. The color of the medium shall be clearly changed within 7 days after inoculation with less than 100 CFU of each of two known strains of *M. pneumoniae* for broth medium for mycoplasma testing I and *M. orale* for broth medium for mycoplasma testing II, respectively, and incubated at 35–37°C.

Mannitol Sodium Chloride Agar Medium

Any dried medium of suitable quality is dissolved according to the direction and sterilized. The pH shall be 7.2–7.6.