

in this example), and elongation at 72°C for 2 minutes. Add drops of mineral oil or suitable equivalent as needed during the reaction to prevent evaporation.

5. Agarose gel electrophoresis

- 1) Mix 10 μL of each of the first stage and second stage PCR products with 2 μL of an appropriate dye as a migration marker, and perform 1% agarose gel electrophoresis.
- 2) Stain the gel with ethidium bromide and take a photograph under UV irradiation.
- 3) The test is judged to be positive if a DNA band is detected.

[An Example of Primer]

For mycoplasma detection

Outer primer

F1: 5'-ACACCATGGGAG(C/T)TGGTAAT-3'

R1: 5'-CTTC(A/T)TCGACTT(C/T)CAGACCCAAGG-CAT-3'

Inner primer

F2: 5'-GTG(G/C)GG(A/C)TGGATCACCTCCT-3'

R2: 5'-GCATCCACCA(A/T)A(A/T)AC(C/T)CTT-3'

() indicates a mixture.

[PCR reaction solution]

	[First stage]	[Second stage]
dNTP solution (each 1.25 mol)	16 μL	16 μL
Primer (10 pmol/ μL)	F1 2 μL	F2 2 μL
Primer (10 pmol/ μL)	R1 2 μL	R2 2 μL
Heat-resistant DNA polymerase (1 U/ μL)	2 μL	2 μL
Reaction buffer solution	68 μL	77 μL
25 mmol/L magnesium chloride hexahydrate	8 μL	8 μL
10-fold buffer solution*	10 μL	10 μL
Sterile distilled water	50 μL	59 μL

*Composition of 10-fold buffer solution

2-amino-2-hydroxymethyl-1,3-propanediol-hydrochloric acid (pH 8.4)	100 mmol/L
Potassium chloride	500 mmol/L
Magnesium chloride hexahydrate	20 mmol/L
Gelatin	0.1 g/L

[Method of cultivating mycoplasma within Vero cells]

- 1) Use at least two cell culture dishes for each of the test sample, positive control and negative control.
- 2) Into each cell culture dish (diameter 35 mm), inoculate 2 mL of the Vero cell suspension (1×10^4 cells per 1 mL) in Eagle's minimum essential medium containing 10 percent bovine calf serum (tested in advance using the PCR method to verify that it does not contain any detectable mycoplasma DNA). Incubate the cultures at $36 \pm 1^\circ\text{C}$ in an atmosphere of air containing 5 percent carbon dioxide for one day.
- 3) Replace the culture media with fresh media, and add 0.5 mL of the test sample (cell culture supernatant) to each of two or more Vero cell culture dishes. Perform the same procedure for the positive (such as 100 CFU or less *M. hyorhinis*) and negative controls.
- 4) Incubate the Vero cell culture dishes for the test sample, positive and negative controls for 3 to 6 days at $36 \pm 1^\circ\text{C}$ in an atmosphere of air containing 5 percent carbon dioxide.

10. pH Test for Gastrointestinal Medicine

In this test, medicine for the stomach and bowels, which is said to control stomach acid, is stirred in a fixed amount of the 0.1 mol/L hydrochloric acid for a fixed duration, and the pH value of this solution is obtained. The pH value of a stomach medicine will be based on the dose and the dosage of the medicine (when the dosage varies, a minimum dosage is used) and expressed in the pH value obtained from the test performed by the following procedure.

Preparation of Sample

Solid medicine which conforms to the general regulations for medicine (the powdered medicine section) can be used as a sample. When the medicine is in separate packages, the content of 20 or more packages is accurately weighed to calculate the average mass for one dose and mixed evenly to make a sample. For granules and similar types in separate packages, among the solid medicine which does not conform to the general regulations for medicine (the powdered medicine section), the content of 20 or more packages is accurately weighed to calculate the average mass for one dose and is then powdered to make sample. For granules and similar types not in separate packages, among solid medicine which does not conform to the general regulations for medicine (the powdered medicine section), 20 doses or more are powdered to make a sample. For capsules and tablets, 20 doses or more are weighed accurately to calculate the average mass for one dose or average mass and then powdered to make a sample.

Liquid medicine is generously mixed to make a sample.

Procedure

Put 50 mL of the 0.1 mol/L hydrochloric acid with the molarity coefficient adjusted to 1.000, or equivalent 0.1 mol/L hydrochloric acid with its volume accurately measured in a 100-mL beaker. Stir this solution with a magnetic stirrer and a magnetic stirrer rotator (35 mm length, 8 mm diameter) at the speed of about 300 revolutions per minute. While stirring, add the accurately weighed one-dose sample. After 10 minutes, measure the pH value of the solution using the pH Determination. The solution temperature should be maintained at $37 \pm 2^\circ\text{C}$ throughout this operation.

11. Plastic Containers for Pharmaceutical Products

Various kinds of plastics are used in the manufacture of containers for pharmaceutical products. Such plastics should not alter the efficacy, safety or stability of the pharmaceutical products. In selecting a suitable plastic container, it is desirable to have full information on the manufacturing processes of the plastic container including the substances added. Since each plastic has specific properties and a wide variety of pharmaceutical products may be stored in containers made from it, the compatibility of plastic containers with pharmaceutical products should be judged for each combination of container and the specific pharmaceutical product to be contained therein. This judgement should be

carried out by verifying that a type sample of the container for the pharmaceutical preparation fulfills the essential requirements, i.e., the design specifications, according to experiments and/or scientific documentation, etc. In addition, the compatibility must be ensured based upon an appropriate quality assurance system.

Furthermore, in introducing a plastic container, it is desirable that proper disposal after use is taken into consideration.

Essential Requirements in Designing Plastic Containers for Pharmaceutical Products

The plastic material for the container should be of high quality. Therefore, recycled plastic materials, which are of unknown constitution, must not be used.

The leachables or migrants from the container should not alter the efficacy or stability of the pharmaceutical products contained therein. In addition, the possible toxic hazards of the leachables or migrants should not exceed a given level. Furthermore, the amounts of leachable or migratable chemical substances, such as monomers and additives, from the containers to the pharmaceutical products contained therein must be sufficiently small from the viewpoint of safety.

The container should have a certain level of physical properties such as hardness, flexibility, shock resistance, tensile strength, tear strength, bending strength, heat resistance and the like, in accordance with the intended usage.

The quality of the pharmaceutical products contained in the container must not deteriorate during storage. For example, in the case of pharmaceutical products which are unstable to light, the container should provide a sufficient level of light shielding. In the case of pharmaceutical products which are easily oxidized, the container material should not allow the permeation of oxygen. In the case of aqueous pharmaceutical products and pharmaceutical products that must be kept dry, the container material should not allow the permeation of water vapor. In addition, care should be taken that the container is impermeable to the solvent in the case of solvents other than water. The concentration of the pharmaceuticals must not be decreased by more than a certain level due to the absorption of the pharmaceuticals on the surface of the container, the migration of the pharmaceuticals into the inside of the material of the container, or the loss of pharmaceuticals through the container. Also, the pharmaceutical products contained therein must not be degraded by an interaction with the material of the container.

The container should not be deformed, should not deteriorate and should not be degraded by the pharmaceutical products contained therein. Unacceptable loss of function of the container should not result from possible high temperature or low temperature or cycles thereof encountered during storage or transportation.

The container should be of a required level of transparency, when it is necessary to examine foreign insoluble matter and/or turbidity of the pharmaceutical products by visual observation.

In the case of pharmaceutical products which must be sterilized, it is required to satisfy the above-mentioned essential requirements of the container after the sterilization if there is a possibility that the quality of the container may change after the sterilization. There should not be any residue or generation of new toxic substances of more than certain risk level after the sterilization. In addition, the container should not have any inappropriate structure and/or

material that might result in any bacterial contamination of the pharmaceutical products contained therein during storage and transportation after sterilization.

Toxicity Evaluation of Container at Design Phase

For design verification, the toxicity of the container should be evaluated. For the toxicity evaluation, it is desirable to select appropriate test methods and criteria for the evaluation, and to clarify the rationales for the selection. The tests should be conducted using samples of the whole or a part of the prototype container. If the container consists of plural parts of different materials, each part should be tested separately. Such materials as laminates, composites, and like are regarded as a single material. To test containers made of such materials, it is recommended to expose the inner surface of the container, which contacts the pharmaceutical products contained therein, to the extraction media used in the tests as far as possible.

The tests required for the toxicity evaluation of the container are different depending upon the tissue to which the pharmaceutical products contained therein are to be applied.

The following tests are required for containers for

- 1) preparations contacting blood:
Acute toxicity test, cytotoxicity test, sensitization test and hemolysis test
- 2) preparations contacting skin or mucous membranes:
Cytotoxicity test and sensitization test
- 3) liquid orally administered preparations:
Cytotoxicity test

It is recommended to conduct the tests in accordance with the latest versions of the standard test methods on medical devices and materials published in Japan and other countries.

Those standard test methods are listed for information:

(A) Selection of Tests

·Guidelines for Basic Biological Tests of Medical Devices and Materials (PAB Notification, YAKU-KI NO.99, June 27, 1995), Principles and selection of tests

·ISO 10993-1: Biological evaluation of medical devices—Evaluation and testing

(B) Acute Toxicity Test

·ASTM F750-82: Standard practice for evaluating material extracts by systemic injection in the mice

·BS5736: Part 3 Method of test for systemic toxicity; assessment of acute toxicity of extracts from medical devices

·USP 24 <88> Biological reactivity tests, in vivo

(C) Cytotoxicity Test

·Guidelines for Basic Biological Tests of Medical Devices and Materials, I. Cytotoxicity Test 10. Cytotoxicity test using extract of medical device or material

·ISO 10993-5: Biological evaluation of medical devices—Tests for cytotoxicity : in vitro methods

·USP 24 <87> Biological reactivity tests, in vitro

(D) Hemolysis Test

·Guidelines for Basic Biological Tests of Medical Devices and Materials, VII. Hemolysis Test

·ISO 10993-4: Biological evaluation of medical devices—Selection of tests for interaction with blood. Annex D

·ASTM F756-82: Standard practice for assessment of hemolytic properties of materials

(E) Sensitization Test

·Guidelines for Basic Biological Tests of Medical Devices and Materials, II. Sensitization Test

·ISO 10993-10: Biological evaluation of medical devices—Tests for irritation and sensitization

Test Results to be Recorded per Production Unit

At the line production phase, it is required to establish the values of acceptable limits on at least the test items mentioned below and to record the test results of each production unit of plastic containers for pharmaceutical products. In addition, it is desirable to clarify the rationales for setting the values of limits. However, these requirements should not be applied to orally administered preparations except liquid ones.

- 1) Combustion Tests: Residue of ignition, heavy metals. If necessary, the amounts of the specified metals (lead, cadmium, etc.)
- 2) Extraction Tests: pH, ultraviolet absorption spectra, potassium permanganate-reducing substances, foaming, non-volatile residue
- 3) Cytotoxicity Test
- 4) Any other necessary tests for the specific container for aqueous infusions.

12. Preservatives-Effectiveness Tests

The purpose of the tests is to assess microbiologically the efficacy of preservatives in a product. The efficacy of the preservatives is assessed by direct inoculation and mixing of the test strains in the product, and further titration of survivals of the test strains with time.

The efficacy, either due to the action of the ingredients themselves or the additives, must be demonstrated for all injections packaged in multidose containers. The efficacy must also be demonstrated for other dosage forms such as otic, ophthalmic and nasal products. Furthermore, it is necessary to demonstrate the efficacy of oral products such as antacids for multiple dosing. Products are divided into two categories for purpose of testing: products made with aqueous bases, and other products. The first category is subdivided into four subtypes depending on the dosage form.

The microorganisms specified for use in the tests should be representatives of those that might be expected to be found in the environment in which the product is manufactured, used or stored. The designated microorganisms should be supplemented by other microorganisms which have contaminated or might contaminate the product and grown on it by their specific properties or by special manufacturing process. Preservatives must not be used solely to pass GMP for drugs or to reduce viable aerobic counts. The preservative itself is a toxic substance, and it must not be added to the product in amounts which might jeopardize the safety of human beings. Considerations must be given to minimizing the amounts of preservative used.

Products and their Categories

The products have been divided into two categories for these tests. Category I includes products made with aqueous bases, and Category II, those made with nonaqueous bases. Oil-in-water emulsions are considered Category I products, and water-in-oil emulsions Category II. Category I is further divided into four subtypes depending on the dosage forms.

Category IA: Injections and other parenterals including otic and ophthalmic products

Category IB: Topically used products applied to mucous surfaces, including liquids for nasal instillation and inhalants

Category IC: Oral products except for antacids made with aqueous bases

Category ID: Antacids (including solid forms of antacids intended for aqueous constitution)

Category II : All the dosage forms listed under Category I made with nonaqueous bases.

Test Microorganisms and Culture Media

The following strains or those considered to be equivalent are used as the test microorganisms.

Escherichia coli ATCC 8739, NCIMB 8545

Pseudomonas aeruginosa ATCC 9027, NCIMB 8626

Staphylococcus aureus ATCC 6538, NCTC 10788

Candida albicans ATCC 10231, NCPF 3179

Aspergillus niger ATCC 16404, IMI 149007

In addition to the five designated strains, strains which might contaminate products and grow on them during the manufacturing process may be used as test microorganisms. (For example, a suitable strain of the osmophilic yeast *Zygosaccharomyces rouxii* may be used with products containing high concentrations of sugars.) Single-strain challenges rather than mixed cultures should be used. The test strains can be harvested by growth on solid agar medium or liquid medium.

Cultures on agar plate media: Inoculate each of the five test strains on the surface of agar plates or agar slants. For growth of bacteria, use Soybean-Casein Digest Agar Medium, and for yeasts and moulds, use Sabouraud Agar, Glucose-Peptone (GP) Agar or Potato Dextrose Agar Medium. Incubate bacterial cultures at 30°C to 35°C for 18 to 24 hours, the culture of *C. albicans* at 20°C to 25°C for 48 hours and the culture of *A. niger* at 20°C to 25°C for one week. Harvest the cells aseptically using a platinum loop, etc. Suspend in sterile physiological saline or 0.1% peptone water and adjust the viable cell count to about 10⁸ microorganisms per mL. To harvest *A. niger*, suspend in sterile physiological saline or 0.1% peptone water containing 0.05 w/v% of polysorbate 80 with the viable cell count adjusted to about 10⁸ microorganisms per mL. Use these suspensions as the inocula.

Liquid cultures: After culturing each of the four strains except for *A. niger* in a suitable medium, remove the medium by centrifugation. Wash the cells in sterile physiological saline or 0.1% peptone water and resuspend them in the same solution with the vegetative cell or spore count of the inoculum adjusted to about 10⁸ per mL.

When strains other than the five listed above are cultured, select a culture medium suitable for growth of the strain concerned. The cell suspension may also be prepared by a method suitable for that strain. Immediately before use titrate the viable cell count of the inocula obtained by the agar plate or liquid culture method. Calculate the viable cell count per mL (g) of the product immediately after inoculation from the viable cell count obtained. Do not allow the suspension to stand at room temperature for more than one hour.