the adsorbate gas. Determine the integral value and calculate the desorbed volume by using this value.

Repeat the measurements with at least three different gas mixtures, varying the ratio of the gases in the mixture by operating the gas mixing unit. For the dynamic flow method, the mix ratio of gases is the relative pressure for the surface area calculation. The volume of gas desorbed is typically used as the volume of gas adsorbed.

Method 2 Volumetric Method

In the volumetric method, the test powder is placed in a sample container with a known volume and the volume of gas adsorbed is determined from the change in pressure associated with the adsorption of gas on the surface of sample powder. Nitrogen is typically used as the adsorbate gas, and a gas such as krypton is preferred for samples with a small specific surface area.

Equipment

The test equipment typically comprises a sample container, a gas supply unit, a calibrated volume section, a vacuum pump, a pressure gauge, a vacuum meter, and a Dewar vessel. The sample container is made from glass, and can be easily attached and detached from the main unit so that pretreatment of the sample can be performed easily. The Dewar vessel is used to hold the liquid nitrogen used to cool the sample container. The void volume indicates the total volume of space in the system that is occupied by the adsorbate gas, including the sample container after the powder sample is placed, in it, the pressure gauge, and the connected system. The void volume should be kept as small as possible to allow measurement of the volume of the gas adsorbed with higher accuracy.

Procedure

Precisely weigh the tared sample container. Place a quantity of the test powder having a total surface area of at least 1 m^2 in the sample container. Perform pretreatment to remove any gases and vapors that have been physically adsorbed onto the sample surface, by outgassing the sample under reduced pressure. Heating may also be employed as long as there is no effect on the physical or chemical properties of the sample. After the pretreatment is completed, precisely weigh the sample container with the sample and subtract the mass of the tared container measured previously, to obtain the mass of the test powder.

Take a fixed quantity of the adsorbate gas and introduce it into the sample container that has been chilled by immersion in liquid nitrogen. The adsorbate gas is adsorbed on the powder sample. The pressure decreases until gas/solid adsorption reaches to a new equilibrium. The volume of gas adsorbed is calculated from the difference between the volume of adsorbate gas that was introduced and the volume of unadsorbed gas remaining in the void volume. The void volume is measured by using helium either before or after the adsorption measurements are performed.

For the multiple-point BET method, calculate the specific surface area by repeating 3 measurements or more of the volume adsorbed, under a relative equilibrium pressure of the adsorbate gas in the range from 0.05 to 0.30. For the single-point method, make one measurement of the volume adsorbed, under a relative pressure of close to 0.30.

Equipment Calibration

The equipment calibration is performed by using α-alumina for specific surface area determination. Measure the specific surface area of the α-alumina according to the specified method. The specific surface area obtained must be within the range specified for the reference standard of α-alumina. The α-alumina for specific surface area determination must be pre-processed according to the specified method.

54. Sterility Test

The test is a method to establish the presence or absence of viable microorganisms (bacteria and fungi) using the defined culturing method. Unless otherwise indicated, the test is carried out by I. Membrane filtration method or II. Direct transfer method. The test should be conducted by personnel well trained in aseptic techniques and the results should be interpreted by personnel having basic knowledge of general microbiology.

Water, reagents, test solutions, equipment, materials and all other requisites for the test should be presterilized. All the operations should be conducted with proper precautions to maintain sterility in an aseptic facility or in aseptic equipment controlled under Grade A conditions as described in the chapter entitled "Microbiological Evaluation of Processing Areas for Sterile Pharmaceutical Products".

Media, rinsing fluids and their preparation

Thioglycollate medium I for sterility test and soybean-casein digest medium are used, unless otherwise specified. When it is difficult to use thioglycollate medium I for sterility test due to turbidity or viscosity of samples, thioglycollate medium II for sterility test can be used, provided it is heated on a water bath just prior to use and incubated under anaerobic conditions. Other products of suitable quality yielding similar formulations may be used according to the indications on the label.

(1) Thioglycollate medium I for sterility test

L-Cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose	5.0 g
Yeast extract	5.0 g
Casein peptone	15.0 g
Sodium thioglycollate	0.5 g
Resazurin solution (1 in 1000)	1.0 mL
Water	1000 mL

(pH after sterilization: 6.9 - 7.3)

Mix all the ingredients and heat until solution is effected. If necessary, adjust the pH of the solution with sodium hydroxide TS so that, after sterilization, it will show 6.9 to 7.3. Filter while hot through moistened filter paper, if necessary. Mix thoroughly, place the required volume of the medium in suitable containers, which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a color change at the end of the incubation period, and sterilize in an autoclave using a validated process. Store the medium at room temperature, protected from light. Do not use the medium when its fluidity shows any change owing to evaporation of water during storage or when the upper one-third or more has acquired a pink color.

(2) Thioglycollate medium II for sterility test

L-Cystine	0.5 g
Sodium chloride	2.5 g
Glucose	5.0 g
Yeast extract	5.0 g
Casein peptone	15.0 g
Sodium thioglycollate	0.5 g
Water	1000 mL

(pH after sterilization: 6.9 - 7.3)

The methods for preparation follow those of thioglycollate medium I for sterility test.

(3) Soybean-casein digest medium

Casein peptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogenphosphate	2.5 g
Glucose	2.5 g
Water	1000 mL

(pH after sterilization: 7.1 - 7.5)

Mix all the ingredients and heat until solution is effected. If necessary, adjust the pH of the solution with sodium hydroxide TS so that, after sterilization, it will show 7.1 to 7.5. Filter through filter paper, if necessary, place the required volume of the medium in suitable containers, and sterilize in an autoclave using a validated process. Store the medium at room temperature, protecting from light.

(4) Rinsing fluids

Animal tissue or casein peptone	1.0 g
Water	1000 mL

(pH after sterilization: 6.9 - 7.3)

Dissolve animal tissue or casein peptone in water and adjust the pH of the solution so that, after sterilization, it will show 6.9 to 7.3. To rinsing fluid to be used for pharmaceutical products containing an antimicrobial agent and medicines containing lecithin or oil, add Polysorbate 80 at 0.1% to 1.0% as necessary. Filter through filter paper, if necessary, place the required volume of the fluid in suitable containers, and sterilize in an autoclave using a validated process.

Growth promotion test

Whenever media are prepared or commercially prepared media are purchased, test the media for their growth-promotion qualities. Separately inoculate containers of each medium with 10 to 100 viable microorganisms of each of the strains listed in Table 1 or other strains considered to be equivalent to these strains, and incubate at the temperature specified for the sterility test. Each of the test organisms should show clearly visible growth in all inoculated media within 3 days for bacteria and within 5 days for fungi. Media stored in tight containers can be used for one year, provided that they are tested for growth promotion within three months of the time of use and that the requirements are met.

Table 1. Microorganisms for growth promotion test

Medium	Test microorganisms	Incubation conditions
Thioglycollate medium I for sterility test	*Staphylococcus aureus* (ATCC 6538, IFO 13276)*1 *Pseudomonas aeruginosa* (ATCC 9027, IFO 13275)*2 *Clostridium sporogenes* (ATCC 11437, IFO 14293)*3	Aerobic
Thioglycollate medium II for sterility test	*Clostridium sporogenes* (ATCC 11437, IFO 14293)*3	Anaerobic
Soybean-casein digest medium	*Bacillus subtilis* (ATCC 6633, IFO 3134, JCM 2499) *Candida albicans* (ATCC 10231, IFO 1594, JCM 2085) *Aspergillus niger* (ATCC 16404, IFO 9455)	Aerobic

*1 An alternative strain is *Bacillus subtilis* (ATCC 6633, IFO 3134, JCM 2499).
*2 An alternative strain is *Micrococcus luteus* (ATCC 9341, IFO 12708).
*3 An alternative strain is *Bacteroides vulgatus* (ATCC 8482, IFO 14291).

Table 2. Number of items to be taken from the lot

Number of items in the lot	Minimum number of items to be taken from the lot
Injections	
Not more than 100 containers	10% or 4 containers, whichever is greater
More than 100 but not more than 500 containers	10 containers
More than 500 containers	2% or 20 containers, whichever is less
For large-volume products (More than 100 mL)	2% or 10 containers, whichever is less
Ophthalmic and other non-injectable products	
Not more than 200 containers	5% or 2 containers, whichever is greater
More than 200 containers	10 containers
Bulk solid products	
Up to 4 containers	Each container
More than 5 containers but not more than 50 containers	20% or 4 containers, whichever is greater
More than 50 containers	2% or 10 containers, whichever is greater

Number of items to be tested

Items to be used for the test are taken from the lot according to an appropriate sampling plan prepared by referring to the numbers specified in Table 2.

Validation tests for bacteriostasis and fungistasis

Before conducting the sterility test for an article, examine whether the inoculated sample has antimicrobial activity or not. Once a lot of the medicine has been tested, the medicine subsequently produced through the same process and having the same ingredients does not need to be tested lot by lot.

Membrane filtration method: Filter the test specimen as shown in I-5 and rinse the membrane. Then filter the rinsing fluid containing approximately 10 to 100 viable microorganisms of each of the strains shown in Table 1 or other equivalent strains. This filter will serve as the test filter. Repeat the rinsing procedure on another filter that has not been exposed to the test specimen. This filter will serve as the positive control. Incubate these filters for not more than 7 days under the condition indicated in I-6 and compare the growth. If the growth of any test organism in the test containers is not observed, is not visually comparable to that in the positive control, or is poor, it is considered that an antimicrobial substance was adsorbed on the membrane. In such a case, the antimicrobial activity should be suppressed by suitable means such as replacement of the membrane filters with less adsorptive ones, increase of the amount of rinsing fluid, or addition of a suitable inactivating agent to the rinsing fluid. If five rinses each with 100 mL of rinsing fluid, containing a suitable amount of surfactant, per filter membrane fail to remove the antimicrobial activity, proceed with the sterility test.

Direct transfer method: Inoculate approximately 10 to 100 viable microorganisms of each of the strains shown in Table 1 or other equivalent strains into the medium containers containing product solution specified in II-3 and the medium containers without product solution. Incubate these containers for not more than 7 days under the conditions indicated in I-6. If the growth of any test organism is not observed in the test containers, is not visually comparable to that in the positive control, or is poor, it is considered that the product possesses antimicrobial activity. In this case, use a suitable inactivating agent which does not affect the growth of microorganisms or increase the volume of medium irrespective of the prescription in II-3 so that no antimicrobial activity remains. If the use of a neutralizing agent or the increase of the volume of the medium is not effective to remove the antimicrobial activity, use the membrane filtration method. For a product to which the membrane filtration method can not be applied, inoculate the amount shown in Table 4 as a minimum quantity.

I. Membrane filtration method

By this method, a test article is filtered through a membrane filter, and the filter is rinsed and incubated by being transferred to a medium or by adding a medium to the filtration apparatus.

I-1. Opening of container

For hermetic containers, disinfect their exterior surfaces with a suitable immediately effective disinfectant, and open them aseptically. Open containers sealed under vacuum after breaking the vacuum with a sterile needle attached to a sterile air filter. When samples are taken from other containers or packages, also take aseptic precautions.

Table 3. Quantities of article for liquid medicines

Container content	Minimum quantity taken from each container for each medium
Less than 1 mL	Whole contents
1 – 10 mL	Half the contents
More than 10 mL	10% of the content or 5 mL, whichever is greater

Table 4. Quantities of article for hydrophobic medicines

Container content	Minimum quantity taken from each container for each medium
Less than 50 mg	Whole contents
50 – 300 mg	Half the contents
More than 300 mg	150 mg

I-2. Preparation of sample solution

A liquid medicine is used, as it is, as the sample solution. In the case of a solid medicine, to be administered after dissolving or suspending, the sample solution is prepared with the provided solvent, isotonic sodium chloride solution or water to give the concentration of use. For a hydrophobic medicine, the sample solution is prepared by using sterile isopropyl myristate that has previously been filtered through a sterilizing membrane filter or by using other solvents not affecting the growth of microorganisms. Warm the sample preparation, if necessary, to a maximum of 44°C for less than 15 minutes.

I-3. Quantities of sample solution to be filtered

For a liquid medicine and for a solid medicine to be administered after dissolving or suspending, take a quantity of the product specified in Table 3. For a hydrophobic medicine, unless otherwise indicated, take a quantity of the product specified in Table 4. For a solid bulk product, take the quantity corresponding to 10 containers of the final product from each bulk container being tested, unless otherwise specified.

I-4. Apparatus for filtration

Use a membrane filter made from suitable material having a diameter of 20 to 50 mm and a nominal pore size of 0.45 μm or smaller. Use a filter funnel sterilizable by the moist heat method or other methods and free from any leakage or back flow when filtration is performed with the membrane in place.

I-5. Procedure

Usually complete the filtration of the sample solution with one or two separate filter funnels. If the sample solution is not readily filterable, it may be further diluted with rinsing fluid and thereafter filtered. Transfer the sample solution to the filter funnel(s), filter, and rinse the membrane(s) with three 100-mL portions of rinsing fluid. Provided the sample does not have antimicrobial activity, the rinsing procedure can be omitted. Employ either of the two methods described below for incubation of the membrane(s).

(1) The processed membrane is aseptically transferred from the apparatus and cut into two equal parts, or half the volume of sample solution is filtered into an entire membrane. Transfer each half of the cut membrane, or each whole membrane in the latter case, into 100 mL of the medium.

(2) After filtration of sample solution into the apparatus to which the membrane filters are fitted, 100 mL of each medium is added to the apparatus itself.

I-6. Culture and observation

Incubate thioglycollate medium I for sterility test at between 30°C and 35°C and soybean-casein digest medium at between 20°C and 25°C for not less than 14 days. Observe the test containers for growth of microorganisms at least once between the fifth and ninth day, two times in total. If the sample makes the medium turbid so that the presence or absence of microbial growth can not be determined readily or in other case of need, transfer suitable portions of the medium to fresh containers of the same medium, incubate the transfer containers at the same temperature for not less than 7 days and examine the medium for growth.

I-7. Interpretation

If no evidence of microbial growth is found as a result of the above-mentioned test, the product tested meets the requirement of the Sterility Test. If microbial growth is found, the product tested fails to meet the requirement of the Sterility Test. However, provided that various factors and/or properties of the contaminant(s) suggest that the sterility test itself was inadequate, the test is repeated. If no evidence of microbial growth is found in the repeat test the product complies with the Sterility Test. If microbial growth is found in the repeat test the product does not comply with the Sterility Test.

II. Direct transfer method

This is the method by which the entire content or a portion of the content of a sample container is transferred directly to the culture medium and incubated. Usually, this method is applied for medicines to which the membrane filtration method can not be applied or for which the application of the direct transfer method, rather than the Membrane filtration method, is rational.

II-1. Opening of containers

Usually, proceed as directed for the Membrane filtration method.

II-2. Preparation of sample solution

Usually, proceed as directed for the Membrane filtration method. In the case of an insoluble medicine, the product is suspended or crushed in a suitable manner and used as a sample.

II-3. Quantities of sample solution to be transferred

For a liquid medicine and for a solid medicine to be administered after dissolving or suspending, unless otherwise specified, take a quantity of the product specified in Table 3. The volume of the sample should be not more than 10% of the volume of the medium. For a hydrophobic medicine, transfer the quantity prescribed in Table 4 according to the amount stated on the label into 200 mL each of thioglycollate medium I for sterility test and soybean-casein digest medium.

II-4. Culture and observation

Proceed as directed for the Membrane filtration method.

II-5. Interpretation

Proceed as directed for the Membrane filtration method.

55. Sulfate Limit Test

The Sulfate Limit Test is a limit test for sulfate contained in drugs.

In each monograph, the permissible limit for sulfate (as SO_4) is described in terms of percentage (%) in parentheses.

Procedure

Unless otherwise specified, transfer the quantity of the sample, directed in the monograph, to a Nessler tube, dissolve it in sufficient water, and add water to make 40 mL. Add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the test solution. Transfer the volume of 0.005 mol/L sulfuric acid VS, directed in the monograph, to another Nessler tube, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions according to the same procedure.

Add 2 mL of barium chloride TS to the test solution and to the control solution, mix well, and allow to stand for 10 minutes. Compare the white turbidity produced in both solutions against a black background by viewing downward or transversely.

The turbidity produced in the test solution is not thicker than that of the control solution.

56. Test for Acid-neutralizing Capacity of Gastrointestinal Medicines

The Test for Acid-neutralizing Capacity of Gastrointestinal Medicines is a test to determine the acid-neutralizing capacity of a medicine, as a crude material or preparation, which reacts with the stomach acid and exercises an acid control action in the stomach. When performing the test according to the following procedure, the acid-neutralizing capacity of a crude material is expressed in terms of the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per g of the material, and that of a preparation is expressed by the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per dose per day (when the daily dose varies, the minimum dose is used).

Preparation of sample

A crude material and a solid preparation which conforms to Powders in the General Rules for Preparations: may be used, without any treatment, as the sample. Preparations in dose-unit packages: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, mix uniformly, and use the mixture as the sample. Granules in dose-unit packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, powder it, and use as the sample. Granules not in dose-unit packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: take not less than 20 doses, powder it, and use as the sample. Capsules and tablets: take not less