（3071）STERILITY TESTS

This chapter has undergone pharmacopeial harmonization. The potential discrepancies between the Pharmacopeial Discussion Group texts and this chapter were considered no impact on the harmonization status.

The following procedures are applicable for determining whether a Pharmacopeia article purporting to be sterile complies with the requirements. Sterility tests is very exacting procedure, where asepsis of the procedure must be ensured for a correct interpretation of results, it is important that personnel be properly trained and qualified.

These procedures are not by themselves designed to ensure that a batch of product is sterile or has been sterilized. This is accomplished primarily by validation of the sterilization process or of the aseptic processing procedures.

When evidence of microbial contamination in the article is obtained by the appropriate Pharmacopeia methods, the results so obtained is conclusive evidence of failure of the article to meet the requirements of the test for sterility, even if a different result is obtained by an alternative procedure.

1. Media

Prepare media for the tests as described below, or dehydrated formulations may be used provided that, when reconstituted as directed by the manufacture or distributor, they meet the requirements of the 1.2.2. Growth Promotion Test.

The following culture media have been found to be suitable for the test for sterility. Fluid Thioglycollate Medium is primarily intended for the culture of anaerobic bacteria. However, it will also detect aerobic bacteria. Soybean–Casein Digest Medium is suitable for the culture of both fungi and aerobic bacteria.

Fluid Thioglycollate Medium I is recommended for sterility testing of clearly visible biological products, and Fluid Thioglycollate Medium II is recommended for turbid or viscous biological products.

1.1. Preparation of Medium

1.1.1. Fluid Thioglycollate Medium I

L-Cystine 0.5 g
Agar (moisture content not exceeding 15%) 0.75 g
Sodium Chloride 2.5 g
Dextrose Monohydrate/Anhydrous 5.5 g
Yeast Extract (water-soluble) 5.0 g
Pancreatic Digest of Casein 15.0 g
Sodium Thioglycollate 0.5 g
or Thioglycolic Acid 0.3 mL
Resazurin Sodium Solution (1 in 1000), freshly prepared 1.0 mL

Purified Water (distilled or deionized water)1000 mL
pH after sterilization: 7.1±0.2.

Mix and heat until solution is effected. If necessary, adjust the pH of the solution with 1 N sodium hydroxide so that after sterilization it will have a pH of 7.1 ± 0.2. Filter while hot through a filter paper, if necessary. Transfer the medium to suitable containers that 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 indicative of oxygen uptake at the end of the incubation period, and sterilize using a validated process. If more than the upper one-third of the medium has a pink color, the medium may be restored once by heating the containers in a water-bath until the pink color disappears. When ready for use, not more than the upper one-third of the medium in a container should have a pink color. Incubate under aerobic conditions.

For products containing a mercurial preservative that cannot be tested by the membrane filtration method, Fluid Thioglycollate Medium incubated at 20˚~25˚ may be used instead of Soybean-Casein Digest Medium provided that it has been validated as described in 1.2.2. Growth Promotion Test.

1.1.2. Fluid Thioglycollate Medium II

Prepare a mixture having the same composition as that of the Fluid Thioglycollate Medium, but omitting the agar and the resazurin sodium solution. Sterilize as directed above. The pH after sterilization is 7.1 ± 0.2. Incubate under anaerobic conditions for the duration of the incubation period.

1.1.3. Soybean–Casein Digest Medium

Pancreatic Digest of Casein 17.0 g
Papaic Digest of Soybean Meal 3.0 g
Sodium Chloride 5.0 g
Dibasic Potassium Phosphate 2.5 g
Dextrose Monohydrate 2.5 g
Purified Water (distilled or deionized water) 1000 mL
pH after sterilization: 7.3±0.2.

Dissolve the solids in the Purified Water, heating slightly to effect a solution. Cool the solution to room temperature, and adjust the pH with 1 N sodium hydroxide so that, after sterilization, it will have a pH of 7.3 ± 0.2. Filter, if necessary to clarify, dispense into suitable containers, and sterilize using a validated procedure. Incubate under aerobic conditions.

1.1.4. Media for Penicillins or Cephalosporins

Where sterility test media are to be used in the 4.2. Direct Transfer Method under 4. Test Procedures, modify the preparation of Fluid Thioglycollate Medium and the Soybean–Casein Digest Medium as follows. To the containers of each medium, transfer aseptically a quantity of β-lactamase sufficient to inactivate the amount of antibiotic in the specimen under test. Determine the quantity of β-lactamase

required to inactivate the antibiotic by using a β-lactamase preparation that has been assayed previously for its penicillin- or cephalosporin-inactivating power. [NOTE—Supplemented β-lactamase media can also be used in the membrane filtration test.]

Alternatively (in an area completely separate from that used for sterility testing), confirm that an appropriate amount of β-lactamase is incorporated into the medium, following either method under 2. Validation Tests for Bacteriostasis and Fungistasis, using less than 100 colony-forming units (CFU) of *Staphylococcus aureus* (see Table 1) as the challenge. Typical microbial growth of the inoculated culture must be observed as a confirmation that the β-lactamase concentration is appropriate.

Table 1. Strains of the Test Microorganisms Suitable for Use in the 1.2.2. Growth Promotion Test and the Method Suitability Test[1]

Medium	Test Microorganisms		Incubate for 5-7 days	
	Microorganism	Suitable strains[2]	Incubation Temperature	Incubation Conditions
Fluid Thioglycollate Medium I	*Staphylococcus aureus*[3] (Aerobic bacteria)	ATCC 6538[2], BCRC 12154	30～35˚	Aerobic
	Pseudomonas aeruginosa[4] (Aerobic bacteria)	ATCC 9027, BCRC 11633		
	Clostridium sporogenes[5] (Anaerobic bacteria)	ATCC 19404, BCRC 11258 ATCC 11437, BCRC 13856		
Fluid Thioglycollate Medium II[6]	*Clostridium sporogenes*[5] (Anaerobic bacteria)	ATCC 19404, BCRC 11258 ATCC 11437, BCRC 13856	30～35˚	Anaerobic
Soybean–Casein Digest Medium	*Bacillus subtilis* (Aerobic bacteria)	ATCC 6633, BCRC 10447	20～25˚	Aerobic
	Candida albicans (Fungi)	ATCC 10231, BCRC 21538		
	Aspergillus brasiliensis (Fungi)	ATCC 16404, BCRC 30506		

[1] Seed-lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed lot.

[2] ATCC 6538 is American Type Culture Collection (ATCC) Number, BCRC12154 is Bioresource Collection and Research Center, FIRDI, ROC (BCRC) Number, and so on.
[3] An alternative to *Staphylococcus aureus* is *Bacillus subtilis* (ATCC 6633, BCRC 10447).
[4] An alternative microorganism is *Kocuria rhizophila (Micrococcus luteus)* ATCC 9341, BCRC 10449.
[5] An alternative to *Clostridium sporogenes,* when a nonspore-forming microorganism is desired, is *Bacteroides vulgatus* (ATCC 8482, BCRC 12903).
[6] Use for sterility test of devices that have tubes with small lumens.

1.2. Suitability Tests

The media used comply with the following tests, carried out before, or in parallel, with the test on the product to be examined.

1.2.1. Sterility of Media

Confirm the sterility of each sterility batch of medium by incubating a portion of the batch at the specified incubation temperature for not less than 14 days or by incubation uninoculated containers as negative controls during a sterility test procedure.

1.2.2. Growth Promotion Test

Test each lot of ready-prepared medium and each batch of medium prepared either with less than 100 viable microorganisms of each of the strains listed in Table 1, and incubate according to the conditions specified. Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed-lot. The media are suitable if a clearly visible growth of the microorganisms occurs. However, the sterility test is considered invalid if the sterility of the media or this 1.2.2. Growth Promotion Test is not successful.

1.3. Storage

1.3.1. Freshly Prepared Media

If prepared media are stored in unsealed containers, they can be for 1 month, provided that they are tested for growth promotion within 2 weeks of the time of use and that color indicator requirements are met. If stored in tight containers, the media can be used for 1 year, provided that they are tested for growth promotion within 3 months of the time of use and that the color indicator requirements are met.

1.3.2. Ready-To-Use Media

Commerically prepared media stored in tight containers may be used provided that the requirements of the sterility of Media and the 1.2.2. Growth Promotion Test are met.

1.4. Diluting and Rinsing Fluids for Membrane Filtration

1.4.1. Fluid A

Preparation

Dissolve 1 g of peptic digest of animal tissue in water to make 1 L, filter or centrifuge to clarify, if necessary, and adjust to a pH of 7.1 ± 0.2. Dispense into containers, and sterilize using a validated process.

Preparation for Penicillins or Cephalosporins

Aseptically add to the above Preparation, if necessary, a quantity of sterile β-lactamase sufficient to inactivate any residual antibiotic activity on the membranes after the solution of the test specimen has been filtered (see 1.1.4. Media for Penicillins or Cephalosporins).

1.4.2. Fluid D

To each L of Fluid A add 1 mL of polysorbate 80, adjust to a pH of 7.1 ± 0.2, dispense into containers, and sterilize using a validated process. Use this fluid for articles containing lecithin or oil, or for devices labeled as “sterile pathway.”

1.4.3. Fluid K

Dissolve 5.0 g of peptic digest of animal tissue, 3.0 g of beef extract, and 10.0 g of polysorbate 80 in water to make 1 L.

Adjust the pH to obtain, after sterilization, a pH of 6.9 ± 0.2. Dispense into containers, and sterilize using a validated process.

2. Validation Tests for Bacteriostasis and Fungistasis

Before instituting the use of a sterility test procedure for an article, ensure that any bacteriostatic and fungistatic activity inherent in the article to be tested does not adversely affect the reliability of the test and that the test procedure to be instituted is otherwise suitable for use with the article. Prepare dilute cultures of bacteria and fungi from the strains of microorganisms listed in Table 1 to obtain a final concentration of microorganisms in the product of less than 100 CFU per mL. Repeat the test method for each microorganism used. [NOTE—If the procedure or media specified under 2.1. Method I does not eliminate the antimicrobial activity, alternative media or neutralizers can be used as long as they are capable of overcoming bacteriostasis or fungistasis.

2.1. Method I

Procedure—Method I is used for validation of bacteriostasis and fungistasis by the 4.1. Membrane Filtration Method. Filter the specified quantity of the test specimen, using the same number of containers per single filter unit or canister as will be used in the sterility test. If necessary, rinse the membrane with a minimum of three 100-mL portions of the appropriate rinsing fluid. Inoculate the

final rinse with less than 100 CFU. Repeat the rinse procedure on another filter that has not been exposed to the specimen under test. This filter will serve as the positive control. Place the filter or filter halves into 100-mL volumes of the specified test medium, or add the specified medium to the canister containing the membrane filter. Repeat the procedure for the appropriate microorganisms and media specified in Table 1, and incubate the containers at the appropriate temperature for not more than 7 days.

Interpretation—If the growth of each test organism in the test containers is visually comparable to the growth in the positive control, use the same amounts of article, number and volume of rinses, and medium when conducting the sterility test. If the growth of the test organisms in the test containers is not visually comparable to that in the positive control, the amount of article used is bacteriostatic or fungistatic. Repeat the test, using a larger number of rinses. Changes in the type of membrane filter used and in the use of neutralizing agents, if available, may reduce the antimicrobial effect of the article (see Interpretation under 2.2. Method II). If five rinses, each of about 500 mL, fail to neutralize the antimicrobial residue on the test filter membrane, proceed with the sterility test.

2.2. Method II

Procedure—Method II is used for the validation of bacteriostasis and fungistasis by the 4.2. Direct Transfer Method. Inoculate two containers of each sterility test medium with less than 100 colony-forming units, using the volume of medium (see Table 3) for each appropriate microorganism specified in Table 1. Add the specified portion of the article under test to one of the inoculated containers of each medium. The other inoculated container is the positive control. Repeat the procedure for each appropriate microorganism, and incubate the containers at the appropriate temperature for not more than 7 days.

Interpretation—If the growth of the test organisms in the test container is not visually comparable to that of the inoculated control container, the article is bacteriostatic or fungistatic. The use of a sterile neutralizing agent, such as polysorbate 80, lecithin, azolectin, or β-lactamase, may be appropriate. If a neutralizing agent is not effective, establish suitable increased volumes of medium. Use the smallest volume of medium in which the growth of test microorganisms in the presence of the article is not adversely affected. [NOTE—If the medium volume is increased to 2000 mL and antimicrobial activity is still present, proceed with the sterility test using the 2000 mL of medium.] Volumes of medium greater than 2000 mL may be needed for testing medical devices, to permit complete immersion of the device.

3. Test for Sterility of the Product to be Examined

3.1. Number of Articles to be Tested

Unless otherwise specified elsewhere in this chapter or in the individual monograph, test the number of articles specified in Table 2. If the contents of each article are of sufficient quantity (see Tables 3 and 4), they may be divided so that equal appropriate portions are added to each of the specified media.

[NOTE—Perform sterility testing employing two or more of the specified media.] If each article does not contain sufficient quantities for each medium, use twice the number of articles indicated in Table 2.

3.2. Opening Articles

Great care must be exercised when opening an article so that the sample to be tested for sterility is not contaminated by microorganisms present on the exterior of the container. The exterior surfaces of ampuls and closures of vials and bottles must be cleansed with a suitable decontaminating agent, and the containers must be placed in an environment that prevents recontamination of the exterior surfaces. If the vial contents are packaged under vacuum, admit sterile air by means of a suitable sterile device, such as a needle attached to a membrane filter holder containing a sterilizing grade filter. For articles such as purified cotton, gauze, surgical dressing, sutures, and related Pharmacopeial articles, decontaminate the outer package, and open the package or container aseptically.

3.3. Quantity of Article

When using the 4.1. Membrane Filtration Method unless otherwise specified elsewhere in this chapter or in the individual monograph, use whenever possible the entire contents of each container, but not less than the quantities specified in Tables 3 and Table 4. When using the 4.2. Direct Transfer Method, use the quantities, indicated in Tables 3 and 4.

Table 2. Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

Number of Items in the Batch[1]	Minimum Number of Items (containers/articles) to be Tested for Each Medium (unless otherwise justified and authorized)[2]
Parenteral preparations	
Not more than 100 containers	10% or 4, whichever is the greater
More than 100 but not more than 500 containers	10
More than 500 containers	2% or 20, whichever is less
For large-volume parenterals	2% or 10, whichever is less
Antibiotic solids	
Pharmacy bulk packages (<5 g)	20
Pharmacy bulk packages (≥5 g)	6
Bulks and blends	See *Bulk solid products*
Ophthalmic and other noninjectable preparations	
Not more than 200 containers	5% or 2, whichever is the greater
More than 200 containers	10

If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral use.	
Catgut and other surgical sutures for veterinary use	2% or 5 packages, whichever is the greater, up to a maximum total of 20 packages
Not more than 100 articles	10% or 4, whichever is greater
More than 100, but not more than 500	10
More than 500 articles	2% or 20, whichever is less
Bulk solid products	
Up to 4 containers	Each container
More than 4 containers, but not more than 50 containers containers	20% or 4, whichever is greater
More than 50 containers	2% or 10, whichever is greater

[1] If the batch size is unknown, use the maximum number of items prescribed.

[2] If the contents of one container are enough to inoculate the two media, this column gives the number of containers needed for both the media together.

Table 3. Minimum Quantity to be Used for Each Medium [1]

Quantity per Container	Minimum Quantity to be Used (unless otherwise justified and authorized) [2]	Minimum Volume of Each Medium (mL)	
		Used for direct transfer of volume taken from each container[3]	Used for membrane or half membrane representing total volume from the appropriate number of containers
Liquids			
Less than 1 mL	The whole contents of each container	≧ 15	100
1–40 mL	Half the contents of each container, but not less than 1 mL	≧ 50	100
Greater than 40 mL, and not greater than 100 mL	20 mL	200	100
Greater than 100 mL	10% of the contents of the container, but not less than 20 mL	≧ 200	100

Antibiotic liquids	1 mL	—	100
Insoluble preparations, creams, and ointments to be suspended or emulsified	Use the contents of each container to provide not less than 200 mg	—	100

[1] Constitue powder products according to the manufacturer's instructions, and then treat as liquid products.

[2] When using the technique of membrane filtration, use, whenever possible, the whole contents of the container, but not less than the quantities indicated.

[3] The volume of the product is not more than 10% of the volume of the medium, unless otherwise prescribed.

Table 4. Minimum Quantity to be Used for Each Medium[1]

Quantity per Container	Minimum Quantity to be Used[1] (unless otherwise justified and authorized)	Minimum Volume, in mL, of Each Medium (mL)	
		Direct transfer[2]	Membrane Filtration
Solids			
Less than 50 mg	The whole contents of each container	200	100
50 mg or more, but less than 300 mg	Half the contents of each container, but not less than 50 mg	200	100
300 mg–5 g	150 mg	200	100
Greater than 5 g	500 mg	200	100
Surgical dressing/cotton/gauze (in packages)	100 mg per package	200	100
Sutures and other individually packaged single-use material	The whole device	≦ 2000	—
Other medical devices	The whole device, cut into pieces or disassembled	≦ 2000[3]	—
Catgut and other surgical sutures for veterinary use	3 sections of a strand (each 30-cm long)	2000	—

[1] When using the technique of membrane filtration, use, whenever possible, the whole contents of the

container, but not less than the quantities indicated.
[2] The volume of the product is not more than 10% of the volume of the medium, unless otherwise prescribed.
[3] Unless the device is bulky and more 2000 mL is needed to submerge the device in the medium.

3.4. Volume of Medium

Unless otherwise specified elsewhere in this chapter or in the individual monograph, the volume of medium used in the test is not less than the volume indicated in Table 3 or Table 4, whichever applies. [NOTE—The final volume used, however, must be adjusted according to the results of the 2. Validation Tests for Bacteriostasis and Fungistasis.]

4. Test Procedures

The test may be carried out using the technique of Membrane Filtration or by Direct Inoculation of the Culture Medium with the product to be examined. Appropriate negative controls are included. The technique of membrane filtration is used whenever the nature of the product permits; that is, for filterable aqueous preparations, for alcoholic or oily preparations, and for preparations miscible with, or soluble in, aqueous or oily solvents, provided these solvents do not have an antimicrobial effect in the conditions of the test.

If the membrane filtration technique is unsuitable, use the Direct Inoculation of the Culture Medium method under 3. Test for Sterility of the Product to be Examined. All devices, with the exception of Devices with Pathways Labeled Sterile, are tested using the Direct Inoculation of the Culture Medium method.

4.1. Membrane Filtration Method

4.1.1. Apparatus

Use membrane filters having a nominal pore size not greater than 0.45 μm, in which the effectiveness to retain microorganisms has been established. Cellulose nitrate filters, for example, are used for aqueous, oily, and weakly alcoholic solutions; and cellulose acetate filters, for example, are used for strongly alcoholic solutions. Specially adapted filters may be needed for certain products (e.g., for antibiotics).

The technique described below assumes that membranes about 50 mm in diameter will be used. If filters of a different diameter are used, the volumes of the dilutions and the washings should be adjusted accordingly. The filtration apparatus and membrane are sterilized by appropriate means. The apparatus is designed so that the solution to be examined can be introduced and filtered under aseptic conditions: it permits the aseptic removal of the membrane for transfer to the medium, or it is suitable for carrying out the incubation after adding the medium to the apparatus itself.

4.1.2. Sample Preparation

(1) Liquids Miscible with Aqueous Vehicles

If appropriate, transfer a small quantity of a suitable, sterile diluent such as Fluid A (see 1.4. Diluting and Rinsing Fluids for Membrane Filtration) onto the membrane in the apparatus and filter. The diluent may contain suitable neutralizing substances and/or appropriate inactivating substances, for example, in the case of antibiotics.

Transfer the contents of the container or containers to be tested to the membrane or membranes, if necessary, after diluting to the volume used in the 2. Validation Tests for Bacteriostasis and Fungistasis with the chosen sterile diluent, but using not less than the quantities of the product to be examined prescribed in *Tables 2* and *3*. Filter immediately. If the product has antimicrobial properties, wash the membrane not less than three times by filtering through it each time the volume of the chosen sterile diluent used in the 2.1. Method I under 2. Validation Tests for Bacteriostasis and Fungistasis. Do not exceed a washing cycle of five times 100 mL per filter, even if during method suitability it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity. Transfer the whole membrane to the culture medium or cut it aseptically into two equal parts, and transfer one half to each of two suitable media. Use the same volume of each medium as in the 2. Validation Tests for Bacteriostasis and Fungistasis. Alternatively, transfer the medium onto the membrane in the apparatus. Incubate the media for not less than 14 days.

(2) Liquids Immiscible with Aqueous Vehicles

Proceed as directed for Procedure under Liquids miscible with aqueous vehicles. If the substance under test is a viscous liquid or suspension and is not adaptable to rapid filtration, aseptically add a sufficient quantity of the appropriate diluting fluid to the pooled specimen prior to filtration to increase the flow rate.

If the substance under test contains lecithin or oil and has inherent bacteriostatic or fungistatic properties or contains a preservative, use Fluid D, and proceed as directed for 2.1. Method I under 2. Validation Tests for Bacteriostasis and Fungistasis, but exclude inoculation of the final rinse with challenge organisms.

(3) Ointments and Oils Soluble in Isopropyl Myristate

Dissolve not less than 100 mg from each of 20 units (or 40 units if the contents are not sufficient for each medium) in 100 mL of isopropyl myristate that previously has been rendered sterile by filtration through a sterilizing membrane filter.[NOTE—Warm the sterile solvent, and if necessary the test material, to a maximum of 44° just prior to use].Swirl the flask to dissolve the ointment or oil, taking care to expose a large surface of the material to the solvent. Filter this solution promptly following dissolution, keeping the filter membranes covered with the solution throughout the filtration for maximum efficiency of the filter. Wash the membranes with two 200-mL portions of Fluid D, then wash with 100 mL Fluid A. Treat the test membranes as directed under Liquids Miscible with Aqueous Vehicles, except that the medium used contains 1 g of polysorbate 80 per liter.

If the substance under test contains petrolatum, use Fluid K, moistening the membranes with about 200 μL of the fluid before beginning the filtration. Keep the membranes covered with the prepared

solution throughout the filtration operation for maximum efficiency of the filter. Following filtration of the specimen, wash the membranes with three 100-mL volume of Fluid K. Treat the test membranes as directed in the previous paragraph.

(4) Prefilled Syringes

For prefilled syringes without attached sterile needles, expel the contents of each syringe into one or two separate membrane filter funnels or into separate pooling vessels prior to transfer. If a separate sterile needle is attached, directly expel the syringe contents as indicated above, and proceed as directed for Liquids Miscible with Aqueous Vehicles. Test the sterility of the needle, using 2.2. Method II under 2. Validation tests for Bacteriostasis and Fungistasis.

(5) Solids For Injection Other Than Antibiotics

Constitute the test articles as directed on the label, and proceed as directed for Liquids Miscible with Aqueous Vehicles or Liquids Immiscible with Aqueous Vehicles, whichever applies. [NOTE—If necessary, excess diluent can be added to aid in the constitution and filtration of the constituted test article.]

(6) Antibiotic Solids for Injection

①Pharmacy Bulk Packages, <5 g—From each of 20 containers, aseptically transfer about 300 mg of solids, into a sterile 500-mL conical flask, dissolve in about 200 mL of Fluid A, and mix; or constitute, as directed in the labeling, each of 20 containers and transfer a quantity of liquid or suspension, equivalent to about 300 mg of solids, into a sterile 500-mL conical flask, dissolve in about 200 mL of Fluid A, and mix. Proceed as directed for Liquids Miscible with Aqueous Vehicles or Liquids Immiscible with Aqueous Vehicles, whichever applies.

②Pharmacy Bulk Packages, ≥5 g—From each of 6 containers, aseptically transfer about 1 g of solids into a sterile 500-mL conical flask, dissolve in about 200 mL of Fluid A, and mix; or constitute, as directed in the labeling, each of 6 containers and transfer a quantity of liquid, equivalent to about 1 g of solids, into a sterile 500-mL conical flask, dissolve in about 200 mL of Fluid A, and mix. Proceed as directed for Liquids Miscible with Aqueous Vehicles.

(7) Antibiotic Solids, Bulks, and Blends

Aseptically remove a sufficient quantity of solids from the appropriate amount of containers (see *Table 2*), mix to obtain a composite, equivalent to about 6 g of solids, and transfer to a sterile 500-mL conical flask. Dissolve in about 200 mL of Fluid A, and mix. Proceed as directed for Liquids Miscible with Aqueous Vehicles.

(8) Sterile Aerosol Products

For fluid products in pressurized aerosol form, freeze the containers in an alcohol-dry ice mixture at least at –20° for about 1 hour. If feasible, allow the propellant to escape before aseptically opening the container, and transfer the contents to a sterile pooling vessel. Add 100 mL of Fluid D to the pooling vessel, and mix gently. Proceed as directed for Liquids Miscible with Aqueous Vehicles or Liquids Immiscible with Aqueous Vehicles, whichever applies.

(9) Devices with Pathways Labeled Sterile

Aseptically pass not less than 10 pathway volumes of Fluid D through each device tested. Collect the fluids in an appropriate sterile vessel, and proceed as directed for Liquids Miscible with Aqueous Vehicles or Liquids Immiscible with Aqueous Vehicles, whichever applies.

In the case of sterile, empty syringes, draw sterile diluent into the barrel through the sterile needle, if attached, or through a sterile needle attached for the purpose of the test, and express the contents into a sterile pooling vessel. Proceed as directed above.

4.2. Direct Transfer Method

Sample Preparation (see 3.1. Number of Articles to be Tested).

4.2.1. Nonfilterable Liquids

Agitate the containers and aseptically withdraw, from a sufficient number of units, the volumes for each medium, as indicated in Tables 2 and 3. Mix each test specimen with the appropriate medium, but do not aerate excessively. Proceed as directed under Procedure.

4.2.2. Ointments, Oils, and Nonfilterable Liquids Insoluble in Isopropyl Myristate

Select 20 units, assign them to 2 groups of 10 units, and treat each group as follows. Aseptically transfer 100 mg, or the entire contents if less than 100 mg, from each of the 10 units to a flask containing 200 mL of a sterile, aqueous vehicle capable of dispersing the test material homogeneously throughout the fluid mixture. If the contents exceed 200 mg, a 100-mg portion may be removed for each medium, with a total of 10 units to be tested. [NOTE—Before initiating use of a given dispersing agent, test the dispersing agent in the concentration to be used to ascertain that it has no antimicrobial effect using the 4. Test Procedures set forth in 2.2. Method II under 2. Validation Tests for Bacteriostasis and Fungistasis.] 1 Mix a 20-mL aliquot of each fluid mixture so obtained with 200 mL of each medium, and proceed as directed under Procedure.

4.2.3. Catgut and other Surgical Sutures for Veterinarian Use

Use for each medium not less than the quantities of the product prescribed in Tables 2 and 4. Open the sealed package using aseptic precautions, and remove three sections of the strand for each culture medium. Carry out the test on three sections, each 30-cm long, which have been cut off from the beginning, the center, and the end of the strand. Use whole strands from freshly opened cassette packs. Transfer each section of the strand to the selected medium. Use sufficient medium to cover adequately the material to be tested (20 mL to 150 mL).

4.2.4. Solids

Transfer a quantity of the product in the form of a dry solid (or prepare a suspension of the product by adding sterile diluent to the immediate container), corresponding to not less than the quantity indicated in Tables 2, 3, and 4. Transfer the material so obtained to 200 mL of Fluid Thioglycollate

Medium, and mix. Similarly, transfer the same quantity to 200 mL of Soybean–Casein Digest Medium, and mix. Proceed as directed above.

4.2.5. Purified Cotton, Gauze, Surgical Dressings, and Related Articles

From each package of cotton, rolled gauze bandage, or large surgical dressings being tested, aseptically remove two or more portions of 100- to 500-mg each from the innermost part of the sample. From individually packaged, single-use materials, aseptically remove the entire article. Immerse the portions or article in each medium, and proceed as directed above.

4.2.6. Sterile Devices

Articles can be immersed intact or disassembled. To ensure that device pathways are also in contact with the media, immerse the appropriate number of units per medium in a volume of medium sufficient to immerse the device completely, and proceed as directed above.

For catheters where the inside lumen and outside are required to be sterile, either cut them into pieces such that the medium is in contact with the entire lumen or fill the lumen with medium, and then immerse the intact unit.

For extremely large devices, immerse those portions of the device that are to come into contact with the patient in a volume of medium sufficient to achieve complete immersion of those portions.

5. Incubation Conditions

Unless otherwise specified elsewhere in this chapter or in the individual monograph, incubate for not less than 14 days at 30~35˚ for the Fluid Thioglycollate Medium or at 20~25˚ for the Soybean-Casein Digest Medium regardless of the method used for sterility testing.

At intervals during the incubating period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portions (each not less than 1 mL) of the medium to fresh vessels of the same medium, and then incubate the original and transfer vessels for not less than 4 days.

Shake cultures containing oily products gently each day. However, when Fluid Thioglycollate Medium is used for the detection of anaerobic microorganisms, keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

6. Interpretation of Test Results

When performing the sterility test in a clean room or clean zone, the article meets the requirements of the test for sterility when no microbial growth is observed. When microbial growth is observed and confirmed microscopically, the article dose not meet the requirements of the test for sterility. However, if the microbial growth can be without a doubt ascribed to faulty aseptic techniques or materials used in conducting the sterility testing procedure, the test is invalid and must be repeated. If microbial

growth is not observed, the article tested meets the requirements of the sterility test. If microbial growth is observed and confirmed microscopically, the article tested dose not meet the requirements of the sterility.