

January 24, 1996

Lee T. Grady, Ph.D.
Division of Standards Development, USP-NF
12601 Twinbrook Parkway
Rockville, MD 20852

Dear Dr. Grady:

Enclosed are PDA's comments regarding proposed changes to General Chapter <71> Sterility Tests. The proposal appeared in the May-June 1995 Pharmacopeial Forum, pages 595-609.

PDA supports USP's decision to clarify this section and offers the enclosed comments to that end.

Please contact Russell E. Madsen, Vice President Scientific and Technical Affairs if there are any questions.

Sincerely,

Edmund M. Fry

Enclosure

STERTEST

PDA Comments
USP <71> Sterility Tests
***Pharmacopeial Forum*, Volume 21, Number 3**
pp. 595-609

In August 1995, PDA formed a Task Force to review the proposed revision to the USP Sterility Test, <71> **Sterility Tests**, published in the May-June 1995 *Pharmacopeial Forum*, pages 595 to 609). The Task Force, comprised of representatives from numerous pharmaceutical companies and contract testing laboratories, supports the USP's Committee of Revision desire to add clarity and detail to this important section.

We believe the Committee of Revision's efforts have been largely successful. However, there are sections of the proposal that need further clarification or revision to ensure that sterility tests are meaningful and not unnecessarily burdensome. For example:

- Testing the entire contents of 40 units will require large amounts of β -lactamase inactivating agent and will require for some larger antibiotic powder configurations as much as 1240 grams of product, significantly increasing the cost of the sterility test without appreciably increasing its sensitivity.
- No retest is permitted for products tested in an isolator under any circumstances. This seems to be an overly rigid requirement and does not allow for the unlikely, but not unforeseeable, situation where it can be proven that a malfunctioning isolator resulted in a false positive result.
- Invalidated tests must be repeated using twice the number of samples used in the original test. This provision does not significantly increase the sensitivity of the sterility test.
- *Bacillus subtilis* is replaced with *Micrococcus luteus* for growth promotion testing of Fluid Thioglycollate Medium, and *Aspergillus niger* is added to the growth promotion testing of Soybean-Casein Digest Medium. This is inconsistent with some other pharmacopeias and international regulations.
- The recommendation to store all sterility test media at 2° to 8°C has not been shown to be necessary and may result in unnecessary expense resulting from construction of refrigerated storage facilities.

The following comments and recommendations are offered from the standpoint of improved harmonization with existing international compendia, regulations and guidelines. Such harmonization is paramount if pharmaceutical manufacturers are to realize lower costs and increased compliance accruing from a set of scientifically valid, harmonized standards.

- Comment #1

<71> STERILITY TEST

"Pharmacoepial articles are to be tested by *Test Procedures Using Membrane Filtration* , unless it is shown that this method is unsuitable for a given article, in which case *Test Procedures for Direct Transfer to Test Media* is to be used."

For small batch sizes, only a single unit is required for testing in each medium. In these cases the use of membrane filtration rather than direct transfer will add unwanted aseptic manipulation, especially if manipulation of the membrane is required.

- Recommended Change

<71> STERILITY TEST

"Pharmacoepial articles are to be tested by *Test Procedures Using Membrane Filtration* , unless it is shown that this method is unsuitable for a given article or requires a sufficiently greater amount of aseptic manipulation than the *Test Procedures for Direct Transfer to Test Media*, in which case *Test Procedures for Direct Transfer to Test Media* may be used."

- Comment #2

Media

FLUID THIOGLYCOLLATE MEDIUM

"Sterilize in an autoclave using a validated cycle."

Media

SOYBEAN-CASEIN DIGEST MEDIUM

"Sterilize in an autoclave using a validated cycle."

The incorporation of "validated cycle" in this section is inconsistent with all other test section present in the USP XXIII. A footnote should be added to the this section to refer the reader to *Sterilization and Sterility Assurance of Compendial Articles* <1211>.

- Recommended Change

Media

FLUID THIOGLYCOLLATE MEDIUM

"Sterilize in an autoclave."¹

Media

SOYBEAN-CASEIN DIGEST MEDIUM

"Sterilize in an autoclave."¹

¹ Media employed in sterility testing must be sterilized using a validated autoclave cycle. See *Sterilization and Sterility Assurance of Compendial Articles* <1211> for guidance in establishing the validity of the sterilization procedure.

- Comment #3

Media

SUPPLEMENTED MEDIA

"Preparation of Media for Penicillin or Cephalosporin Class of Antibiotics - For sterility test media used in the *Test Procedures for Direct Transfer to Test Media*, modify the preparation of *Fluid Thioglycollate Medium* and *Soybean-Casein Digest Medium* as follows. To the containers of each medium, aseptically transfer a quantity of β -lactamase sufficient to inactivate the amount of antibiotic in the article under test. Determine the appropriate quantity of β -lactamase by using a β -lactamase that has been assayed previously for its penicillin- or cephalosporin- inactivating power.

Alternatively (in an area completely separate from that used for sterility testing), to confirm that an appropriate amount of β -lactamase is incorporated into the medium, use the following procedure. Add to a container of *Supplemented Fluid Thioglycollate Medium* an amount of penicillin and cephalosporin, equivalent to the amount of antibiotic in the article under test. Inoculate the container with 1-mL of a 1:1000 dilution of an 18 to 24 hour culture of *Staphylococcus aureus* (ATCC#29737) in *Fluid Thioglycollate Medium*, and incubate for 24 hours at 30° to 25°. Typical microbial growth must be observed as a confirmation that the β -lactamase is in an appropriate concentration."

The temperature for incubation is incorrect and should be stated as 30° to 35°. Additional clarity should be added to indicate that typical growth of the inoculated organism must be observed. The section on qualifying the β -lactamase should also be expanded to indicate that a bioassay should be performed by the manufacturer or testing laboratory prior to use.

- Recommended Change

"Preparation of Media for Penicillin or Cephalosporin Class of Antibiotics - For sterility test media used in the *Test Procedures for Direct Transfer to Test Media*, modify the preparation of *Fluid Thioglycollate Medium* and *Soybean-Casein Digest Medium* as follows. To the containers of each medium, aseptically transfer a quantity of β -lactamase sufficient to inactivate the amount of antibiotic in the article under test. Determine the appropriate quantity of β -lactamase by using a β -lactamase that has been assayed previously for its bio-activity by the manufacturer or testing laboratory for its penicillin or cephalosporin inactivating power.

Alternatively (in an area completely separate from that used for sterility testing), to confirm that an appropriate amount of β -lactamase is incorporated into the medium, use the following procedure. Add to a container of *Supplemented Fluid Thioglycollate Medium* an amount of penicillin and cephalosporin, equivalent to the amount of antibiotic in the article under test. Inoculate the container with 1-mL of a 1:1000 dilution of an 18 to 24 hour culture of *Staphylococcus aureus* (ATCC#29737) in *Fluid Thioglycollate Medium*, and incubate for 24 hours at 30° to 35°. Typical microbial growth of the inoculated organism must be observed as a confirmation that the β -lactamase is in an appropriate concentration."

- Comment #4

In the Media Suitability section, Table 1 refers to a list of test microorganisms used for both the growth promotion and the bacteriostasis and fungistasis test. The substitution of *Micrococcus luteus* for *Bacillus subtilis* for testing Fluid Thioglycollate Medium and Alternative Thioglycollate Medium, and the addition of *Aspergillus niger* to the test for Soybean-Casein Digest broth will require unnecessary revalidation effort at firms which manufacture many different sterile products. These changes work against method harmonization among the BP, EP, and the Australian Therapeutic Goods Act, which allow alternative choices for test organisms. To work towards method harmonization, alternative organism choices should be incorporated so that many international compendial requirements can be met.

- Recommended Change

To allow for harmonization the following changes in Table 1 are suggested.

For Thioglycollate Medium

Primary Organisms	Alternative Organisms
<i>Bacillus subtilis</i> ATCC 6633	<i>Micrococcus luteus</i> ATCC 9341 <i>Staphylococcus aureus</i> ATCC 6538P
<i>Candida albicans</i> ATCC 10231	<i>Candida albicans</i> ATCC 2091
<i>Clostridium sporogenes</i> ATCC 11437	<i>Clostridium sporogenes</i> ATCC 19404 <i>Bacteroides vulgatus</i> ATCC 8482

For Soybean-Casein Digest Medium

Primary Organisms	Alternative Organisms
<i>Bacillus subtilis</i> ATCC 6633	<i>Staphylococcus aureus</i> ATCC 6538P <i>Micrococcus luteus</i> ATCC 9341
<i>Candida albicans</i> ATCC 10231	<i>Candida albicans</i> ATCC 2091 <i>Aspergillus niger</i> ATCC 16404

- Comment #5

Media

STORAGE OF MEDIA

"If freshly prepared media are not used within 2 days, store them in the dark, preferably at a temperature between 2° and 8°, but never greater than 25°. If prepared media are stored in unsealed containers, they can be used for one month, provided they are tested for growth promotion within one week of the time of use and if color indicator requirements are met. If stored in suitable sealed containers, the media can be used for one year, provided they are tested for growth promotion within 3 months of the time of use and if the color indicator requirements are met."

The media utilized in the USP sterility test method are very stable at temperatures between 2° and 25°C. The change in storage temperature to indicate that the preferred temperature is 2° to 8°C could result in some companies having to design and build new refrigerated storage areas, which to now have not been necessary.

Examples of what is considered to be a sealed container should be added for clarity.

- Recommended Change

Media

STORAGE OF MEDIA

"If freshly prepared media are not used within 2 days, store them in the dark, between 2 ° and 25 °. If prepared media are stored in unsealed containers, they can be used for one month, provided they are tested for growth promotion within one week of the time of use and if color indicator requirements are met. If stored in suitable sealed containers (e.g. screw cap bottles or vials), the media can be used for one year, provided the growth promotion and color indicator requirements for media stored in unsealed containers are met."

- Comment #6

Media

STORAGE OF MEDIA

"**Commercially Ready-to-use Media** - The sterility test facility must have the test procedures and results that support extension of the time period from that stated above, before the *Growth Promotion Test* is repeated."

The intent of this section is confusing. Commercially prepared media does not typically have the manufactured date on the label, only the expiration date. There is no reason to believe the requirements for commercially ready-to-use media should differ from media prepared in-house.

- Recommend Change

Media

STORAGE OF MEDIA

Delete the section **Commercially Ready-to-use Media**.

- Comment #7

Bacteriostasis and Fungistasis Test

MEMBRANE FILTRATION METHOD

"Procedure - Filter the specified quantity of the article using the same number of filters as will be used in the sterility test. Rinse the membrane with a minimum of three 100-mL portions of the appropriate rinsing fluid. Inoculate the final rinse with approximately 10 to 100 viable microorganisms. Repeat the rinse procedure on another filter that has not been exposed to the article under test. This filter will be the positive control. Place the filter or filter halves into 100-mL volumes of the specified test medium. Repeat the procedure for all the microorganisms and media listed in Table 1, and incubate the containers at the appropriate temperature for not less than 7 days."

This section indicates that if multiple filters are used than the bacteriostasis and fungistasis testing is to be performed using the same number of filters used in the test. For a test in which the tested article is split between 6 filters each going into a separate media vessel this would indicated that 6 filters would be required, when performing the bacteriostasis and fungistasis test for each required organisms. This requirement has no additional value over performing the bacteriostasis and fungistasis test using the amount of article to be tested per membrane. In the section there is no description on how the bacteriostasis and fungistasis testing is to be performed for articles not requiring the use of a rinsing fluid. This should be included.

- Recommended Change

Bacteriostasis and Fungistasis Test

MEMBRANE FILTRATION METHOD

"Procedure - Filter the specified quantity of the article to be tested per membrane through the appropriate filter type to be used in the sterility test. If required, rinse the membrane with a minimum of three 100-mL portions of the appropriate rinsing fluid inoculating the final rinse with less than 100 viable microorganisms. If rinsing is not required, inoculate the product directly prior to filtration. Repeat this procedure using another filter that has not been exposed to the article under test. This filter will be the positive control. Place the filter or filter halves, if the method used requires the filters to be cut, into 100-mL volumes of the specified test media. Repeat the procedure for all the microorganisms and media listed in Table 1, and incubate the containers at the appropriate temperature for not less than 7 days or until growth is evident."

- Comment # 8

Bacteriostasis and Fungistasis Test

MEMBRANE FILTRATION METHOD

"Interpretation - If the growth of each test organism in the test article containers is visually comparable to that in the positive control, use the same amounts of article and medium when conducting the sterility test. If the growth of the test organism in the test article containers is not visually comparable to that in the positive control, the amount of article used is bacteriostatic or fungistatic. A sterile neutralizing agent (e.g., polysorbate 80, lecithin, azolectin, penicillinase, etc.) can be used to overcome the stasis condition. If a neutralizing agent is not available, establish, as directed below, suitable amounts of media to be used.

Repeat the test set forth above, using the specified amount of article and larger volumes of medium to determine the ratio of article to medium in which growth of the test organisms is not adversely affected. Dilute the article in 100-mL increments up to a maximum of 1000 mL. If the specific amount of article is bacteriostatic or fungistatic in 1000-mL of the medium, use the specified amount of the article in 1000-mL in the sterility test."

The meaning of "visually comparable" is ambiguous. This term should be clarified to indicate that growth in the positive control and test vessel should be comparable in terms of the time required for growth and the resulting turbidity. This clarification will also provide better method harmonization with the EP and BP.

There is no indication that additional filters, filters composed other materials, or larger rinse volumes, can be used to eliminate a product's bacteriostatic and/or fungistatic properties.

The last paragraph of this section appears to be misleading. The use of larger quantities of media is an appropriate method for reducing product concentrations in the Direct Transfer Method. For the Membrane Filtration Method the appropriate means for reducing product concentrations used for each filter would be the use of additional filters.

- Recommended Change

Bacteriostasis and Fungistasis Test

Membrane Filtration Method

"Interpretation - If the growth of each test organism in the test article containers is visually comparable to that in the positive control in terms of time required for growth and

turbidity, use the same amounts of article and medium when conducting the sterility test. If the growth of the test organism in the test article containers is not visually comparable to that in the positive control, the amount of article used is bacteriostatic or fungistatic. The use of sterile neutralizing agents (e.g., polysorbate 80, lecithin, azolectin, penicillinase, etc.), alternative filters types which reduce the binding of the test article, multiple filters, and/or larger rinse volumes can be used to overcome the stasis condition. For the Direct Transfer Method if a neutralizing agent is not available, establish, as directed below, suitable amounts of media to be used.

Repeat the test set forth above, using the specified amount of article and larger volumes of medium to determine the ratio of article to medium in which growth of the test organisms is not adversely affected. Dilute the article in 100-mL increments up to a maximum of 1000 mL. If the specified amount of article is bacteriostatic or fungistatic in 1000-mL of the medium, use the specified amount of the article in 1000-mL in the sterility test."

- Comment #9

General Procedure

"Pharmacopeial articles are to be tested for sterility by the membrane filtration technique. Where this procedure has been shown to be unsuitable for an article, direct transfer to the test media can be used."

To eliminate redundancy in the sterility test section it is suggested that the first paragraph under General Procedure be removed. This statement is a repeat of the information presented in the first paragraph of the Sterility Test section.

- Recommended Change

Remove Paragraph

- Comment #10

General Procedure

PREPARATIONS FOR TESTING AND INCUBATION PERIOD

"**Quantity** - Unless otherwise specified in the individual monograph, the number of units is 20 per medium for a total of 40 per test. For small batches comprising 40 units or less, test 1 unit per medium; for those comprising more than 40 units, test 10% of the batch up to a maximum of 20 per medium."

The change to indicate that the entire contents of 40 units are to be tested with the

exception of small lots does not adequately recognize the vast diversity of product types and container volumes present in the industry. This proposal represents a substantial move away from harmonization with the international community, which the USP has indicated to be one of its goals. In addition, this change has no scientific value as the increase in sample size has no appreciable statistical impact on the test's sensitivity, which remains extremely low. Increasing the number of containers and volumes tested will not increase the assurance that a sterile product has been produced. This type of assurance can only come from quality systems and quality operations being in place during manufacture of the batch. The proposed change will also require unnecessary revalidation efforts at firms manufacturing many different sterile products.

- Recommended Change

- General Procedure**

- PREPARATIONS FOR TESTING AND INCUBATION PERIOD

- "**Quantity** - Unless otherwise specified in the individual monograph, for liquid articles, use not less than the volumes of articles and the number of containers per medium specified in Table 2. If the contents are of sufficient quantity, they may be divided such that portions are added to each of the two specified media. If each container does not contain sufficient volume for both media, use double the number of containers.

- For solid articles, take a quantity of the product in the form of a dry solid (or of a solution or a suspension of the product prepared by adding sterile diluent to the immediate container), corresponding to not less than 300 mg (150 mg per medium) from each container being tested, or the entire contents if each contains less than 300 mg of solids.

- For small batches comprising 40 units or less, test 1 unit per medium."

TABLE 2
 Quantities for Liquid Articles

Container Content (mL)	Minimum volume taken from each container for each medium	No. of containers per medium
Less than 10	1 mL, or entire contents if less than 1 mL	20
10 to less than 50	5 mL	20
50 to less than 100	10 mL	20
50 to less than 100, intended for intravenous administration	One half contents	20
100 to 500	One half contents	20
over 500	250 mL	20

- Comment #11

General Procedure

PREPARATION FOR TESTING AND INCUBATION PERIOD

"**Opening Units** - Great care must be exercised when opening a unit 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 ampules and closures of vials and bottles must be cleansed with a suitable decontaminating agent,..."

The entire exterior surface of the unit should be decontaminated with a suitable agent, not just the closures of vials and bottles, when the entire unit is placed into the aseptic field.

- Recommended Change

General Procedure

PREPARATION FOR TESTING AND INCUBATION PERIOD

"**Opening Units** - Great care must be exercised when opening a unit so that the sample to be tested for sterility is not contaminated by microorganisms present on the exterior of the container. The entire exterior surfaces of ampules, vials, and bottles must be cleansed with a suitable decontaminating agent,..."

- Comment #12

General Procedure

PREPARATION FOR TESTING AND INCUBATION

"**Volume of Unit** - Unless otherwise specified in the individual monograph, the entire content of each unit is tested."

This section should be deleted per Comment #10.

- Recommended Change

Delete paragraph.

- Comment #13

General Procedure

TESTING FACILITIES AND PERSONNEL

"**Training and Qualification of Personnel** - An appropriate training program includes the testing of a product known to be sterile as part of the qualification of the analyst prior to conducting sterility tests. A positive rate not exceeding 0.5% of those samples tested would qualify a technician for sterility testing. Simulate all of the manipulations carried out in either a clean room environment or in an isolator system that are used in the sterility testing of the samples. It is also good practice to review periodically the number of test samples found positive for each technician in the routine testing of samples, and, if warranted, requalify the technician for sterility testing."

Training programs generally do not have a quantitative method for assessment of aseptic manipulation control. Having specific false positive rates for individual testers is difficult when a tester is only used infrequently or the number of tests performed by a laboratory is low. One false positive requires many tests to reduce the false positive rate and may cause manufactures to eliminate occasional testing personnel. Training of sterility personnel is presently covered in chapter <1211> of USP 23 (p. 1980). The information presented should be included in this section.

- Recommended Change

General Procedure

TESTING FACILITIES AND PERSONNEL

"Training and Qualification of Personnel - The sterility testing procedure should be performed by individuals having a high level of aseptic technique proficiency. Test performance records for these individuals should be maintained. Appropriate, known-to-be sterile, finished articles should be employed periodically as negative controls as a check on the reliability of the test procedure. Preferably the technician performing the test should be unaware that they are testing negative controls. Of these tests, a false positive frequency not exceeding 0.5% is desirable."

- Comment #14

General Procedure

SAMPLE PREPARATION

"Liquids Miscible with Aqueous Vehicles - Aseptically transfer the entire contents of each unit tested either directly into one or two separate membrane filter funnels or to separate pooling vessels prior to transfer. Each membrane represents the entire contents of 20 units. If the membrane is cut in half, each half of the membrane must represent the entire contents of 20 units. Immediately pass each specimen through the filter with the aid of vacuum or pressure. If only one membrane is used, aseptically remove the membrane from the holder, cut the membrane in half, and immerse half of the membrane in each of the specified medium. If two membranes are used, immerse one membrane into each of the specified medium. If a closed system is used, fill one of the membrane canisters with each of the specified medium. [Note - During all manipulations, avoid aeration of the *Fluid Thioglycollate Medium* .]

If the product under test has inherent bacteriostatic or fungistatic properties or contains a preservative, using *Fluid A*, follow the procedure as indicated under the *Membrane Filtration Method* in the *Bacteriostatic and Fungistatic Test*. "

This section does not account for the use of multiple membrane filters or rinsing of the membrane to eliminate the bacteriostatic and/or fungistatic properties associated with various product types. The last paragraph requires that the sterility test is to be performed as outlined in the Membrane Filtration Method in the Bacteriostatic and Fungistatic Test. This implies that control organisms are to be added to the third or final rinse when performing sterility testing.

- Recommended Change

General Procedure

SAMPLE PREPARATION

"Liquids Miscible with Aqueous Vehicles - Aseptically transfer the volumes for both media, as indicated in Table 2, either directly into one or two separate membrane filter funnels or to separate sterile pooling vessel(s) prior to transfer. The required volumes from not less than 20 containers are thus represented by one membrane, or membrane half, transferred to each medium. [NOTE—In some cases, where the liquid is highly viscous and not readily filterable through one or two membranes, or has bacteriostatic and/or fungistatic properties, more than two filtration assemblies may be used. In such cases the article under test must be divided between the filtration units such that the required volumes from not less than 20 containers are tested for each medium type.] Immediately pass each specimen through the filter with the aid of vacuum or pressure. If the product is bacteriostatic and/or fungistatic, rinse the membrane(s) with three 100-mL portions of an appropriate rinsing fluid as determined in the *Bacteriostasis and Fungistasis Test*. If only one membrane is used, aseptically remove the membrane from the holder, cut the membrane in half, and immerse half of the membrane in each specified medium. If two membranes are used, immerse one membrane into each specified medium. If a closed system is used, fill one of the membrane canisters with each of the specified medium. [NOTE—During all manipulations, avoid aeration of the *Fluid Thioglycollate Medium* .]"

- Comment #15

General Procedure

SAMPLE PREPARATION

"Liquids Immiscible with Aqueous Vehicles - Proceed as indicated in the *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, using Fluid D, follow the procedure as indicated under the *Membrane Filtration Method* in the *Bacteriostatic and Fungistatic Test*."

The second paragraph of this section is previously covered under *Liquids Miscible with Aqueous Vehicles* and is therefore redundant. The section also restricts the use of rinsing fluids to Fluid D. For products which contain inherent bacteriostatic and/or fungistatic properties, alternative rinsing fluids may be required to eliminate these

properties.

- Recommended Change

General Procedure

SAMPLE PREPARATION

"Liquids Immiscible with Aqueous Vehicles - Proceed as indicated in the *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."

- Comment #16

General Procedure

SAMPLE PREPARATION

"Ointments and Oils Soluble in Isopropyl Myristate - Dissolve 100 mg from each of 40 units in 100-mL of isopropyl myristate, which previously has been rendered sterile by filtration through a 0.22 μm membrane filter. [NOTE—Warm the sterile solvent, and if necessary the test material, to 44° just prior to use.]"

See Comment #10 regarding sample size. These product types are very difficult to weigh aseptically. The term "about" should be added to allow small variations which may occur in the weighing process. To allow for variations which occur in testing, and for harmonization with the EP, the 44° requirement should be changed to indicate not greater than 40°.

- Recommended Change

General Procedure

SAMPLE PREPARATION

"Ointments and Oils Soluble in Isopropyl Myristate - Dissolve about 100 mg, accurately weighed, from each of 20 units in 100-mL of isopropyl myristate, which previously has been rendered sterile by filtration through a 0.22 μm membrane filter. [NOTE—Warm the sterile solvent, and if necessary the test material, to not greater than 40° just prior to use.]"

- Comment #17

General Procedure

SAMPLE PREPARATION

"Prefilled Syringes - For those prefilled syringes with attached sterile needles, flush the contents of each syringe into one or two separate membrane filter funnels or to separate pooling vessels prior to transfer. If no sterile needle is supplied, attach a sterilized needle, and expel the contents as indicated above; if no sterilized needle is used, expel the contents as indicated above. Then proceed as directed under *Liquids Miscible with Aqueous Vehicles*."

The second sentence indicates that if no sterile needle is supplied the analyst is required to attach a sterile needle to the syringe and expel the contents. This adds an unnecessary manipulative step to the testing process, which has no relevance to the sterility of the product and may result a false sterility test failure.

- Recommended Change

General Procedure

SAMPLE PREPARATION

"Prefilled Syringes - For those prefilled syringes with attached sterile needles, flush the contents of each syringe into one or two separate membrane filter funnels or to separate pooling vessels prior to transfer. If no sterile needle is supplied expel the contents as indicated above. [NOTE—If desired a sterile needle may be attached to the syringe prior to expelling the syringe contents.] Then proceed as directed under *Liquids Miscible with Aqueous Vehicles* ."

- Comment #18

Test Procedures for Direct Transfer to Test Media

"This method of sterility testing is used only if the *Test Procedure Using Membrane Filtration* cannot be applied because of the nature of the product to be tested."

This statement is a repeat of the information presented in the first paragraph of the Sterility Test section.

- Recommended Change

Remove the paragraph.

- Comment #19

Test Procedures for Direct Transfer to Test Media

SAMPLE PREPARATION

"Nonfilterable Liquids - Remove 1 mL, or the entire contents in less than 1 mL, from a sufficient number of units to obtain 10-mL per medium. Mix each 10-mL pooled content with each medium, but do not aerate excessively. Proceed as directed under *Procedure*."

The quantity of product to be tested is 10 mL for each medium for a total of 20 mL, regardless of the product container volume. The number of units tested is not indicated for this group of test articles, whereas a number of units is either stated or implied for the other groups of test articles. This creates a large difference in test sample size between nonfilterable products and filterable products. The potential result of this discrepancy would be to create a substantially different contribution to overall sterility assurance levels by the same USP test on different products. Therefore, the effect of the sample size discrepancy should be minimized.

- Recommended Change

Test Procedures for Direct Transfer to Test Media

SAMPLE PREPARATION

"Nonfilterable Liquids - Aseptically transfer the volumes for each media, as indicated in the Table 2, either directly into the appropriate media or to separate sterile pooling vessel(s) prior to transfer. Mix each sample with the appropriate media, but do not aerate excessively. Proceed as directed under *Procedure*."

- Comment #20

Test Procedures for Direct Transfer to Test Media

SAMPLE PREPARATION

"Ointments and Oils Insoluble in Isopropyl Myristate - Select 40 representative units, assign them to 2 groups of 20 units, and treat each group as follows. Aseptically

transfer 100 mg, or the entire contents if less than 100 mg, from each of the 20 units to a flask containing 200 mL of a sterile, aqueous vehicle capable of dispersing the test material homogeneously throughout the fluid mixture. {} Mix an aliquot of 20 mL of the fluid mixture so obtained with 200 mL of each medium, and proceed as directed under *Procedure*."

The volume of growth media used is not the critical parameter of this test and will be determined in bacteriostasis and fungistasis testing. Therefore, a range of volumes should be adequate for detection of any microorganisms present in the sample. Also, the volume change to 200 mL is not consistent with the recommended 100 mL volume under the section titled "**General Procedure**," Volume of Medium, where ". . . the volume of medium used in the test is not less than 100 mL. [NOTE—The final volume used, however, must be adjusted according to the results of the *Bacteriostasis and Fungistasis Test*.]" In addition to the change in media volume the proposed revisions raises the number of containers to be tested from 20 to 40. Please see Comment #10 regarding this change.

- Recommended Change

"**Ointments and Oils Insoluble in Isopropyl Myristate** - Select 20 representative 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. {} Mix an aliquot of 20 mL of the fluid mixture so obtained with not less than 100 mL of each medium, and proceed as directed under *Procedure*."

- Comment #21

Test Procedures for Direct Transfer to Test Media

SAMPLE PREPARATION

"**Solids** - Transfer a quantity of the product in a form of a dry solid (or prepare a suspension of the product by adding a sterile diluent to the immediate container), corresponding to not less than 300 mg from each of 20 units being tested or the entire contents of each of 20 units, if each contains less than 300 mg of solids. Transfer the material obtained to 200 mL of *Fluid Thioglycollate Medium*. Similarly, transfer the same quantity from 20 other units to 200 mL of *Soybean-Casein Digest Medium*, and proceed as directed under *Procedure*."

The volume of growth media used is not the critical parameter of this test and will

be determined in bacteriostasis and fungistasis testing. Therefore, a range of volumes should be adequate for detection of any microorganisms present in the sample. Also, the volume change to 200 mL is not consistent with the recommended 100 mL volume under the section titled "**General Procedure**," Volume of Medium, where ". . . the volume of medium used in the test is not less than 100 mL. [NOTE—The final volume used, however, must be adjusted according to the results of the *Bacteriostasis and Fungistasis Test*.]" In addition to the change in media volume, the proposed revisions raises the number of containers to be tested from 20 to 40. Please see Comment #10 regarding this change.

- Recommended Change

"**Solids** - Transfer a quantity of the product in a form of a dry solid (or prepare a suspension of the product by adding a sterile diluent to the immediate container), corresponding to not less than 300 mg from each of 20 units being tested (150 mg per medium) or to the entire contents of each of 20 units, if each contains less than 300 mg of solids to not less than 100 mL of *Fluid Thioglycollate Medium* and not less than 100 mL of *Soybean-Casein Digest medium*, respectively, and proceed as directed under *Procedure*."

- Comment #22

Interpretation of Sterility Test Results

"**Sterility Test Results Performed in a Clean Room** - When no microbial growth is observed or confirmed, the article tested meets the requirements of the test for sterility. When microbial growth is observed and confirmed, determine if the microbial growth can be ascribed to faulty aseptic technique or materials used in conducting the sterility testing procedure by reviewing the following:

- a. The data of the microbiological monitoring of the sterility testing facility.
- b. The qualification and periodic testing record of the technician performing the testing.
- c. The testing procedure used during the testing of the article.
- d. The results of the negative controls.
- e. A determination of the identity of the microorganisms (at least to genus) isolated from the test.

If microbial growth is invalidated based on a review of "a-e" above, the test is completely repeated using double the number of units originally tested. If microbial growth is observed and confirmed, the article tested does not meet the requirements of the sterility test.

Sterility Test Results in an Isolator - If microbial growth is observed and confirmed, the article tested fails to meet the requirements of the test for sterility. **DO NOT RETEST.**"

The term "observed" means that turbidity is seen, while the term "confirmed" means that suspected microbial growth has subsequently been demonstrated by an acceptable method. These terms need to be clarified. The listing of possible causes which could invalidate the sterility test is good. However, no list can provide for all circumstances and for that reason the list presented should be viewed as a partial compilation of possible causes, which should be mentioned in this section.

The use of twice the number of containers for a retest where there is an assignable cause for the failure is too stringent and adds little to the detection probability. If an assignable cause is determined, the first test should be considered invalid and a retest performed using the original sample size.

The requirement that no sterility retest be allowed for testing performed in an isolator unit is too stringent and would result in unnecessary rejection of product if the test failure occurred because of the unlikely circumstance of a faulty isolator. If there is convincing evidence the test environment was compromised during the original test, a retest should be allowed.

- Recommended Change

Interpretation of Sterility Test Results

"Sterility Test Results Performed in a Clean Room - When no microbial growth is observed or confirmed, the article tested meets the requirements of the test for sterility. If evidence of microbial growth, such as turbidity, is observed and confirmed by culture, or other acceptable methodology, and a review of the testing process indicates that there is a *clear and determinate* laboratory cause for the positive result, the test may be invalidated. The following areas are to be included in the review:

- a. The data from the microbiological monitoring of the sterility testing facility.
- b. The qualification and periodic testing record of the technician performing the testing.
- c. The testing procedure used during the testing of the article.
- d. The results of the negative controls.
- e. A determination of the identity of the microorganisms (at least to genus) isolated from the test.

If microbial growth is invalidated based on the review, the test is repeated. If microbial growth is not invalidated based on the review the article tested does not meet the requirements of the sterility test and a retest is not allowed.

Sterility Test Results in an Isolator - When no microbial growth is observed or confirmed, the article tested meets the requirements of the test for sterility. If evidence of microbial growth, such as turbidity, is observed and confirmed by culture, or other acceptable methodology, and a review of the testing process indicates that there is a *clear and determinate* laboratory cause for the positive result, the test may be invalidated. The following areas are to be included in the review:

- a. The data from the microbiological monitoring of the isolator.
- b. The isolator operating parameters and control function.
- c. The results of the negative controls.
- d. A determination of the identity of the microorganisms (at least to genus) isolated from the test.

If microbial growth is invalidated based on the review, the test is repeated. If microbial growth is not invalidated based on the review, the article tested does not meet the requirements of the sterility test and a retest is not allowed. [NOTE—In a properly functioning isolator, a retest would be an extremely rare occurrence.]"