

## Activities of the USP Microbiology Subcommittee of Revision During the 1995 - 2000 Revision Cycle

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**ABSTRACT:** This article is a comprehensive review of the published activities of the Microbiology Subcommittee of the USP Committee of Revision for the 1995 - 2000 revision cycle. The activities of this revision cycle were designed to position USP activities in microbiology that will be useful as technology advances. In addition to reviewing the changes accomplished, this article discusses the rationale for many of the changes and some background information on new initiatives underway. Where appropriate, changes in the USP that did not fall under the direct purview of the MCB Subcommittee but of interest to the microbiology community are also discussed.

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### Introduction

This five-year period of 1995 - 2000 has been one of extensive activity and change not only for the United States Pharmacopeia (USP), but also for other regional pharmacopeias and regulatory bodies. Global harmonization, the rise of new product categories, and the impact of improved methodologies have all played a role in the changing climate of microbiology in the USP. This has been an activist revision cycle, and the extensive changes accomplished during this five-year period are testament to the interest taken in these matters. Many of the publications that occurred in 1995 are actually the work of the Subcommittee during the 1990 - 1995 revision cycle. These are included in this review with the acknowledgement of the hard work of the previous Subcommittee, many of whose members also served in the 1995 - 2000 revision cycle.

The Microbiology (MCB) Subcommittee of the United States Pharmacopeial Convention Subcommittee of Revision has a very clearly defined scope of activities. This scope includes General Chapters and Informational Chapters dealing with microbial assays and microbial

control of processes. This scope does not extend to drug or product monographs, which are handled by other Subcommittees, although the Microbiology Subcommittee does support the development of microbial requirements for all monographs. Similarly, antibiotics are handled by a separate subcommittee, while the responsibility for water is shared with the Water and Parenteral Subcommittee (especially in the area of microbiological control of pharmaceutical waters).

The MCB Subcommittee works within the structure of the USP, developing or revising chapters as deemed necessary. It is important to understand the purpose and application of USP in this regard. Firstly, there are two distinct types of standards encompassed by USP: expert standards and guidance documents. USP standards, microbiological methods included, are referee tests that have a scientific as well as a legal standing; they are expert standards, not consensus standards. Secondly, they are not batch release methods nor are they quality control methods. If, for example, a pharmaceutical product does not fulfill the requirements of the monograph, then it is "mislabelled" or "adulterated" and the regulatory agencies can take action based on these two provisions of the Food, Drug, and Cosmetic Act. Given this legal status of compendial tests, changes to any chapter are made only after careful consideration. Similarly, the guidance documents in USP are widely held as a reference guide to industry, and changes to an official chapter are very slowly introduced.

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The revision process of *USP* is designed to provide the maximum opportunity for input into the deliberative process. Interested parties, which may also include members of the Subcommittee, propose a new chapter or changes to current chapters; these proposals are then forwarded to the Subcommittee for review and consideration. Following review, the proposals will then appear in the journal *Pharmacopeial Forum* (*PF*). If the proposal is a new chapter, or if significant changes are being made to an existing chapter, the proposal will appear as a *Pharmacopeial Preview*; otherwise, the proposal is published in the *In-Process Revision* section of *PF*.

After public comments are received the subcommittee reviews the comments and incorporates them, if warranted, into a new chapter proposal. This proposal will appear in *PF* as an *In-process Revision*. Subsequent public comments and revisions to the chapter will continue to appear in *PF* as an *In-process Revision* until the Subcommittee is satisfied that the proposal is ready to become official. At that point, the Subcommittee proposes the chapter for final adoption to the Committee of Revision. If approved, the chapter appears in one of the semi-annual supplements. These supplements are the mechanism used to update the *USP* between the publication of the book every five years. Official revisions appear in the supplement, and supersede the version in the previous publication.

If a significant number of comments are then received on the revised chapter, it will be re-evaluated, and possibly a new round of *In-process Revision* drafts published and considered. Thus the revision process of *USP* is a continuous one, responsive to changing regulatory needs. A summary of the activity is provided in Table 1. However, it is important to note that this process is a reactive one in which silence from the field is interpreted as assent. If no comments are received on a specific proposal as it appears in *PF*, then this is interpreted by *USP* as approval.

In addition to providing a means for communication of new drafts or proposals, the *Pharmacopeial Forum* also provides a forum for workers in the field to publish scientific articles of interest in the *Stimuli for Revision* section of *PF*. During the 1995 - 2000 revision cycle there were several articles published of particular interest to the work of the Microbiology Subcommittee. These *Stimuli* articles are designed to promote discussion of new ideas or provide data to assist the Subcom-

mittee in improving chapters in the *USP* and have been used in the revision process by the MCB Subcommittee. A listing of the relevant articles is provided in Table 2, and each is discussed later in this article.

The MCB Subcommittee has held a number of *USP* Open Conferences that focused on specific microbiological topics. These open conferences are well attended and generate discussion on proposals made or to be made by the MCB Subcommittee. Each conference results in *Proceedings* with specific recommendations that are reviewed and considered carefully by the Subcommittee. In addition, there have been joint meetings, particularly with the other pharmacopeia and with FDA and PDA, which were very useful in providing input for deliberations (see Table 3).

This review will examine the published activity by chapter in numerical order. This number sequence is important in its own right, as there is an underlying structure to the numbering sequence of *USP* Chapters (explained in the *General Notices* section). Chapters numbered less than <1000> contain referee tests that are enforceable by regulatory agencies. Those chapters numbered from <1000> to <1999> are information chapters that are offered as guidance. Those chapters beginning at <2000> are devoted to nutritional supplements and are considered guidance documents. Chapters reviewed in this article include:

- <51> Antimicrobial Preservatives - Effectiveness
- <52> Antimicrobial Effectiveness Testing for Vaccines
- <55> Biological Indicators - Resistance Performance Tests
- <61> Microbial Limit Tests (Microbial Enumeration Tests)
- <62> Microbiological Procedures for Absence of Objectionable Microorganisms
- <71> Sterility Tests
- <85> Bacterial Endotoxins Test
- <1035> Biological Indicators
- <1111> Microbiological Attributes of Nonsterile Pharmaceutical Products
- <1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments
- <1207> Sterile Product Packaging - Integrity Evaluation

- <1208> Sterility Testing - Validation of Isolator Systems
- <1222> Terminally Sterilized Pharmaceutical Products - Parametric Release
- <1227> Validation of Microbial Recovery from Pharmacopeial Articles
- <2021> Microbial Enumeration Tests - Nutritional and Dietary Articles
- <2022> Microbiological Procedures for Absence of Objectionable Microorganisms in Nutritional and Dietary Articles.
- <2023> Microbiological Attributes of Nonsterile Nutritional and Dietary Articles.

### <51> Antimicrobial Preservatives — Effectiveness

The Antimicrobial Effectiveness Test has been an extremely controversial topic for the MCB over the last five years. Within the United States, there has been a great deal of discussion about reducing interlaboratory variability. Internationally, there has been an ongoing debate about the level of antimicrobial effectiveness necessary to safeguard the patient.

Several proposals have been directed towards the goal of reducing the reputed level of interlaboratory variability in the test. The use of the Phenol Coefficient as a method to determine the suitability of the challenge organisms was proposed in 1992 (1). Due to severe concerns over the adequacy and appropriateness of this method, the Subcommittee proposed several changes designed to qualify the stock cultures used in the assay (2, 3), the first of which was proposed for an Antimicrobial Resistance Suitability Test in 1995. On the basis of comments and recommendations made at the USP Open Microbiology Conference in 1996<sup>1</sup>, the MCB Subcommittee resubmitted the previously proposed revision of this general test chapter with substantive changes. The new proposals included the deletion of the *Stock Culture Antimicrobial Resistance Suitability* section, the requirement for a 21-day sampling interval, and the recommendation to, in addition, use microorganisms that have been isolated from the environment. In addition, a new requirement was added to ensure that all stock cultures used were within five passages from the original ATCC stock. This requirement, a component of the Sterility Test since USP 21 (1985), was included to try to establish control over the organisms used in the test. Other changes included renaming some

of the product categories - Category “1D” for antacids appeared as Category 1C for oral products. After lengthy debate over the peculiar requirements of liquid antacids, it was decided that if special requirements were indeed necessary for this product class, these requirements were to be included in the specific antacid monograph. The MCB Subcommittee planned to develop an informational chapter on the Antimicrobial Effectiveness Test, which would deal with a number of issues raised at the January open conference.

This proposal generated a great deal of discussion in the pharmaceutical community, and was the subject of more discussion at the 1996 Inter-Pharm Conference<sup>2</sup>. An *In-process Revision* was published (4) clarifying that products containing antimicrobial preservatives must fulfill the requirements in the chapter; and editorial changes were made to Table 2.

Final editorial changes were presented early in 1997 (5). This version was approved by the U.S. Pharmacopeial Convention and published in the *Eighth Supplement* to USP 23 - NF 18 (p. 1681) effective May 15, 1998 (6).

By this time there was some confusion in the field about the status of the harmonization efforts for both the Antimicrobial Effectiveness Test and the Sterility Test among the pharmacopeias of Europe, Japan, and the U.S. A review of the current status of this effort was published in the Nov. - Dec. 1997 *PF* (7) as a *Stimuli to the Revision Process*. At that time, the test had reached a point where most of the contentious issues had been analyzed, discussed, and considered. International face-to-face meetings of the pharmacopeial experts along with open conferences have resulted in advances in harmonization. However, outstanding among the issues that were not harmonized were the criteria for antimicrobial effectiveness.

Several new concerns were raised at the 1998 USP Open Conference on Microbiology. Among these was the need to delete the requirements for antimicrobial effectiveness testing of products with a nonaqueous base or vehicle. The deletion of this requirement would improve harmonization with the European and Japanese Pharmacopeias. Therefore, a proposed revision was published in Jan. - Feb. of 1999 (8) with this change. No change in status on this proposal has been made to date.

Participants of the 1998 Open Conference also suggested modifications to the testing of preserved antacid products. These modifications included a reduced test preparation concentration between  $1 \times 10^3$  and  $1 \times 10^4$  CFU per mL and proposed effectiveness criteria for antacids, bacteria, molds, and yeast as “no increase” from the initial count at days 14 and 28. These proposed criteria for preserved antacids were based on current product performance. Antacids remain a discussion point as the new revision cycle begins.

#### <52> Antimicrobial Effectiveness Testing for Vaccines

The current general chapter *Antimicrobial Effectiveness Testing* <51> applies to vaccines in multi-use containers. Significant concern was expressed to the USP MCB that, because of their nature and composition, most vaccines could not fulfill the requirements criteria proposed by the European Pharmacopoeia. At the request of interested parties, USP MCB developed a “stand-alone” chapter designed for the testing and evaluation of vaccines and is offering it as a point of departure for international harmonization discussions. This proposed chapter <52> appeared in the May - June 1998 issue of *PF* (9). No further developments for the proposed chapter <52> are planned as the EP is developing different criteria of effectiveness that would apply to vaccines.

#### <55> Biological Indicators - Resistance Performance Tests

At the urging of industry, the USP MCB developed a chapter to assist manufacturers and end-users of biological indicators (BI). This chapter first appeared as a *Pharmacopoeial Preview* in Nov. - Dec. 1994 (10). The USP MCB proposed substantive changes to this proposed new chapter as an *In-process Revision* in the Sept. - Oct. 1996 issue of *PF* (11). The revision proposed for *D Value Determination* was intended to present the calculations in an improved systematic series of equations. These equations incorporated the summation expressions that had confused some readers when presented in the accompanying text. The net result was a more clear presentation of the math behind the technique.

Publication of the official version occurred in the *Sixth Supplement* (12), effective May 15, 1997. This new chapter, now officially a General Chapter of USP 23 - NF 18, caused some confusion. An *In-Process Revision* appeared in the May - June 1999 *PF* (13) that sought to resolve several significant concerns from the

field. Comments received indicated the need to mention *D Value Determination* methods other than the Spearman-Kärber method described in the original chapter. The USP MCB proposed to cite the survival curve method and the Stumbo, Murphy, Cochran method. In addition, discrepancies in the number of biological indicators to be used in the *D Value Determination*, kill times, and survival times were resolved by requiring groups of 10 biological indicators for each type of indicator. Changes to the informational chapter <1035> *Biological Indicators* and also to all BI monographs were proposed elsewhere in this issue of *PF*.

#### <61> Microbial Limit Tests

After considerable discussions, the MCB Subcommittee decided the chapter needed to be divided into two separate general chapters: one dealing with bioburden determinations, the other with the identification of objectionable microorganisms. This proposal to this effect appeared in the Mar - April 1999 *PF* (14), whereby this chapter was to be divided into two chapters: *Microbial Enumeration Tests* <61> and *Microbiological Procedures for Absence of Objectionable Microorganisms* <62>. This proposal did not recommend changes in the basic procedures for microbial enumeration, but the entire chapter was rewritten.

This proposed chapter included four enumeration tests: Total Aerobic Microbial Count, Total Combined Molds and Yeasts Count, Coliform Count and Enterobacterial Count. The Total Aerobic Microbial Count includes membrane filtration, pour plate, and multiple-tube procedures. The Total Combined Molds and Yeasts Count is performed by employing a membrane filtration, pour plate, or spread plate procedure. In the procedure for Coliform Count, Lauryl Tryptose Broth and the most probable number detection are employed. In the Enterobacterial Count Test, Mosaic Enterobacteriaceae Enrichment Broth and the most probable number determination are employed. The limits for the various enumeration tests are specified in the individual monographs; otherwise, the guidance for establishing limits was provided under Microbiological Attributes of Nonsterile Pharmaceutical Products <1111>, a new General Informational Chapter proposed first in this issue of *PF* (Mar - Apr 1999).

This proposed new chapter <61> clearly stated that each nonsterile compendial article need not be tested by all

four enumeration tests. It was expected that Total Aerobic Microbial Count and Total Combined Molds and Yeasts be performed on all nonsterile articles; in addition, depending on the article, either a Coliform Count or an Enterobacterial Count be performed as specified in the individual monograph.

### <62> Microbiological Procedures for Absence of Objectionable Microorganisms

This proposal for a new chapter appeared in the Mar - April 1999 *PF* (15). It described microbiological procedures for demonstrating the absence of objectionable microorganisms in new materials, excipients, drug substances, and nonsterile dosage forms. The procedures for determining the absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Salmonella* species, *Escherichia coli*, *Candida albicans*, and *Clostridium* species were described. However, these are not the only objectionable microorganisms of potential concern in nonsterile pharmacopeial articles. Objectionable microorganisms whose absence needs to be assessed will be specified in individual monographs. In the latest version of the proposed chapter <IIII> *Microbiological Attributes of Nonsterile Pharmaceutical Products*, which also appeared in this issue of *PF*, a decision tree was provided as a guidance for manufacturers and regulators in deciding which objectionable microorganisms have to be absent from a particular pharmacopeial article. Therefore, the USP MCB committed to review the official monographs in view of this guidance to assess the inclusion or deletion of tests for the absence of specific objectionable microorganisms.

Comments generated by the proposals for <61>, <62>, and <IIII> are being evaluated by the subcommittee. In addition, a harmonization initiative has been started with the European Pharmacopoeia (EP) and the Japanese Pharmacopoeia (JP) for the development of these chapters in the three pharmacopoeias.

### <71> Sterility Tests

This chapter (16) has been under extensive revision during this cycle of the MCB. An extensive revision of the USP general chapter was presented under *Pharmacopeial Previews* in the May - June 1995 issue of *PF* (17), and a proposal for a globally harmonized chapter was published in 1995 (18). However, complete agreement on a harmonized chapter was not yet

final. An *In-process Revision* was published in the Sept - Oct 1996 *PF* (19) that reflected the text previously published under *Previews*. This revision also included substantive changes to the chapter made as a result of discussions at the 1996 USP Open Conference on Microbiology and at the Interpharmacopoeial Open Conference in Barcelona, Spain, in February 1996, as well as comments received at USP headquarters. The chapter was essentially harmonized with that of the European Pharmacopoeia published following the Barcelona Meeting. There are some divergences, however, that caused concern on an international level because of the nature of regulatory requirements in the United States versus those in Europe and Japan. The USP *Sterility Tests* chapter is a referee test; therefore additional details on the conductance of the test must be included in chapter <71>.

Most significant of the changes proposed at this time were dealing with media quality and incubation conditions. The *Growth Promotion Test* is performed once when a lot of commercially-prepared dehydrated medium is used, provided that the sterilization process used to sterilize the medium has been validated and that validation parameters are met; whereas the test must be performed for each batch of a medium prepared in-house. Confirmation of the sterility of a batch can be carried out simultaneously while the test is being done. Media storage requirements were changed to between 2° C and 25° C, storage need not be in the dark, and the requirement for sealed containers was changed to *Tight containers* to be consistent with the USP General Notice on *Preservation, Packaging, Storage and Labeling* (20). The microorganisms specified were similar to those in the then-current proposal of the harmonized chapter. The *Validation Test for Bacteriostasis and Fungistasis* used the same microorganisms as those required in the *Growth Promotion Test*, and the proposed maximum volume of medium to be used is 2000 mL. Several of these provisions drew comment, the most notable of which was the concern over-performing growth promotion on every lot of media prepared. Concern from industry was that this could potentially require every autoclave load of liquid media to be tested. The committee debated this point on the basis of the chapter's fundamental purpose as a referee test rather than a product release test. That is to say, the point of the chapter was to provide a method for determining the sterility of a specific sample, rather than to provide a quality control measure. Therefore, it would be rea-

sonable to require sterility testing of every lot. However, due to the widespread use of this test as a product release test this requirement was amended in a later revision to allow for testing only of the manufacturer's lot of dehydrated media. Assuming adequate autoclave validation, lot-to-lot variability was not considered to be a significant risk for the growth-promoting qualities of liquid media.

The Sept. - Oct. 1996 proposal also stated that the minimum number of articles to be tested be related to the number of articles in a given batch, and the quantities of product to be used for each medium are given for liquid products and solid products, including sterile bulk products and medical devices. It was in this issue that the first mention was made of changing the incubation period to 14 days, the exception being products that are sterilized by a validated moist heat process, in which case the period was to be not less than seven days. Also, the requirements for *Interpretation of Sterility Test Results* specify that if a test is invalidated, the same number of units be used in the retest as in the original test (although it should be noted that an invalid test cannot be thought of as a test at all, and so the second attempt is, in effect, the first valid test of that sample). Provisions were also included in this proposal for repeating a test performed in an isolator system where it can be documented that the physical integrity of the isolator has been breached. The specific training requirements in the May - June 1995 version were deleted.

The MCB Subcommittee finalized the modifications to this general test chapter in the Nov. - Dec. 1997 *PF* (21). This "finalized" proposal was the result of the work that was started during the 1990 -1995 revision cycle. Harmonization with the European and Japanese Pharmacopeias was taken into consideration: a side-by-side comparison of this general test chapter with the EP final draft was presented in this issue of *PF* under *Stimuli to the Revision Process* (22). These proposals were implemented in the *Eighth Supplement* to USP 23 - NF18 (23), with an official date of May 15, 1998.

The newly implemented chapter continued to draw concerns. Following the 1998 USP Open Conference on Microbiology, and after a review of a number of comments received, the USP MCB proposed a number of revisions in the Sept - Oct 1998 *PF* (24). Several proposed revisions were made to clarify some ambiguous portions of the text.

In the area of sterility testing of antibiotics, the proposed revisions would have allowed manufacturers of antibiotics to test the sterility of their bulk products in accordance with FDA regulation, 21 CFR 436.20. However, when 21 CFR 436.20 was deleted, the MCB Subcommittee proposed that sterility testing requirements formerly in 21 CFR 436.20 would be incorporated specifically in Chapter <71> Sterility Tests.

A proposal was included to revise the time of incubation of inoculated test media specified under the *Growth Promotion Test* to more closely harmonize with the text of the *European Pharmacopeia* chapter on sterility (25). The EP requires an incubation time of "not more than three days for bacteria and not more than five days for molds." This proposal was made official in the *Tenth Supplement* (26) with an official date of May 15, 1999. Following a microbiology expert's meeting in 1999<sup>4</sup>, the three pharmacopeias developed a harmonized proposal that was acceptable to all concerned. Proposals to harmonize the test are found in both the *PF* (27) and *PharmEuropa* (28).

#### <85> Bacterial Endotoxins Test

The MCB Subcommittee has been extensively involved in this test during the 1990 - 1995 and 1995 - 2000 revision cycles. The changeover from the USP Pyrogen Test to the Bacterial Endotoxins Test (BET) is now complete in the over 650 monographs that include such requirements.

The MCB Subcommittee has completed the harmonization initiative on BET. Final agreement on the harmonized document was completed in September of 1999 and the result has been published in the Jan-Feb 2000 *PF* (29) and will be proposed for inclusion in the Second Supplement of USP 24 with an implementation date of January 1, 2001.

The main feature of the harmonized document is that three types of methods are included: the Gel Clot Method; a Turbidometric method, and a Colorimetric method. The Gel Clot Method will be considered a referee test in case of conflict. The other methods, once validated for a given product, can be used without having to show equivalency to the Gel Clot Method.

## <1035> Biological Indicators

The proposed revisions in the Nov. - Dec. 1994 issue of *PF* (30) for this general information chapter (31) were canceled and replaced by a new streamlined proposal in the July - Aug 1997 *PF* (32). This proposal included a separate section on the performance evaluation of biological indicators by manufacturers and users. Also included were sections in the evaluation of in-house, noncommercial biological indicators and issues related to spore crop preparations, instrumentation for evaluation of resistance performance characteristics, and use of biological indicators for in-process validation.

## <1111> Microbiological Attributes of Nonsterile Pharmaceutical Products

This general information chapter (33) has been under review for several revision cycles. The previous effort to revise this chapter published in July-Aug. 1992 *PF* (34) was subsequently canceled in a *Pharmacopeial Preview* published in the Nov. - Dec. 1996 issue of *PF* (35). On the basis of recommendations of participants at the 1996 USP Open Conference on Microbiology, this revision of the chapter included information on good manufacturing practices with respect to microbial attributes. Information was also included on the factors involved in the development of microbiological monitoring programs, ranging from the design of products to the packaging materials, and including the facilities, equipment, water, and raw materials. These factors were suggested as important microbiological attributes of drug substances and excipients as well as the nonsterile final dosage forms.

A *Total Aerobic Microbial Count* of 1000 colony-forming units per gram or mL and a *Total Combined Yeasts and Molds Count* of 100 colony-forming units per gram or mL was presented as a general index of the relative levels of microbial contamination of raw ingredients, drug substances, and excipients. It was noted that some monographs could require the absence of specific microorganisms or lower microbial counts because of special considerations. These requirements would be specified in the individual monographs following a review of the present *Microbial Limits* requirements in the USP monographs.

These proposals generated a great deal of interest. On the basis of comments received from reviews and rec-

ommendations made during the 1998 USP Open Conference on Microbiology, the MCB Subcommittee decided to present a new version of this chapter. The new proposal, appearing again as a *Pharmacopeial Preview*, was published in the March - April 1999 *PF* (36). The *Sampling* section was expanded in response to several requests. Furthermore, along with the table of target values for microbial counts in nonsterile dosage forms, a new table was included to specify the target values for raw materials, excipients and drug substances. These target values are to be used in case the individual monograph does not specify the particular microbial enumeration limit. When these microorganisms are not specified in the individual monograph, a decision tree added to this chapter is to be used as a guidance in deciding which objectionable microorganisms have to be absent from the particular *Pharmacopeial* article. The Subcommittee will also review the official monographs in view of this guidance to assess the inclusion or deletion of tests for the absence of specific objectionable microorganisms. Discussions with EP and JP on the need for an informational chapter have already been initiated.

## <1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments

The amount of interest this proposed new informational chapter received during the 1995 - 2000 revision cycle was only slightly less than that elicited by the Sterility Tests and Antimicrobial Preservative Efficacy Test proposals. It seemed that a great many people wanted this chapter, but no one wanted any particular version of this chapter. The USP MCB made substantive changes to the version of this proposal that appeared in the March - April 1995 *PF* (37) following the 1995 USP Open Conference<sup>5</sup>. This proposal appeared in the Jan. - Feb. 1997 *PF* (38). The scope of the chapter was clarified, and the suggested frequency of sampling controlled environments modified. The Subcommittee reviewed the arguments for the deletion and retention of the various action levels and decided to include them as information in this chapter.

This proposal was not received with equanimity in the field and many comments were received in response to the proposal. The number and scope of comments indicate a strong interest in this issue and a need for this type of information in the USP. The MCB Subcommittee reviewed all comments and the changes that they felt were appropriate were published in the Nov. - Dec.

1997 *PF* (39). These proposals were implemented in the *Eighth Supplement* to USP 23 - NF 18 (40), with an official date of May 15, 1998.

This revision was not yet complete, however, as significant changes were proposed for the newly revised chapter within a year of its adoption. The May - June 1999 *PF* (41) contained a proposal that expanded the scope of this information chapter to include isolator environments and the use of controlled environments for aseptically manufactured sterile drugs. The proposed revisions introduced guidelines for product contact surfaces and critical zone surfaces and expanded the discussion on surface monitoring, especially for Class 100 environments where aseptic processing is performed. Several other issues are clarified, including Alert and Action levels. Needless to say, these changes have stimulated discussion on the topic. An *Ad hoc* group composed of members of the Subcommittee and members of an industrial group has been formed to provide information to the subcommittee for inclusion in the next proposed revision.

#### **<1207> Sterile Product Packaging - Integrity Evaluation**

A new proposed informational chapter that appeared as a *Pharmacoepial Preview* in the Nov. - Dec. 1997 *PF* (42) dealt with the topic of container closure integrity testing. This proposed informational chapter provided guidance on integrity testing throughout the life cycle of the product including initial development, routine manufacturing, and shelf-life stability assessments. It also described different physical and microbiological testing methods that might be employed. This test should provide some measure of the ability of the package to withstand microbial ingress, and be reflected in the physical characteristics deemed acceptable to the finished product.

To date there has been little comment on this proposal. As there is no obvious objection from the field, this proposed chapter will be moved to the *In-process Revision* section of a later *PF*.

#### **<1208> Sterility Testing - Validation of Isolator Systems**

The first publication of this proposed new informational chapter appeared as a *Pharmacoepial Preview* in the

Nov - Dec 1997 *PF* (43). This proposal was modified in the Jan - Feb 1999 issue of *PF* (44) based on discussions held at the 1998 USP Open Conference. The major change is that sterilization of the interior of the isolator requires the elimination of all viable bioburden. The revised proposal also indicated that the isolator does not need to meet US Federal Standard 209E Class 100 requirements during operation, and that there were no requirements for air velocity or air exchange rate.

Several minor issues were clarified in this new proposal. Isolators need not be installed in a clean environment, although limited access to nonessential staff was recommended. Modified sections on *Sterilization Cycle Verification* and *Sterilization Cycle Development* under *Operational Qualification* addressed the use of biological indicators, aeration of the isolator enclosure following *Sterilization Validation* under *Performance Qualifications* and on *Maintenance of Asepsis within the Isolator Environment* were also expanded.

#### **<1222> Terminally Sterilized Pharmaceutical Products - Parametric Release**

A new proposed informational chapter that appeared as a *Pharmacoepial Preview* in the Nov. - Dec. 1997 *PF* (45) reviewed the issues of parametric release; validation of sterilization, the microbiology control program, physicochemical indicators and integrators, the need for a change control system, and different release procedures. There are relatively few methods of sterilization, and each was discussed in turn; moist heat, ethylene oxide, and radiation.

To date there has been little comment on this proposal and so it will be forwarded to the *In-process Revision* section of a later *PF*.

#### **<1227> Validation of Microbial Recovery from Pharmacoepial Articles**

This informational chapter first appeared as a *Pharmacoepial Preview* in the Nov. - Dec. 1996 *PF* (46). It was written in response to specific requests from the field for guidance on validation issues with *Microbial Limits*, *Antimicrobial Preservative Effectiveness*, and *Sterility Tests* and includes a strong recommendation for statistical analysis of microbiology data. A draft of this new general information chapter was first pre-

sented at the January 1996 USP Open Conference where input was sought from the participants. The recommendations of participants were reviewed by the Subcommittee and changes were made in the original document as a result of the deliberations before publication as a *Pharmacopeial Preview* (44). The proposal generated little comment from the field and was forwarded to *In-process Revision* with a few editorial changes (47). Some comments were received from the field at this stage of the development of this chapter. These concerns were discussed by the MCB Subcommittee and a new proposal was developed. The new proposal was published in the Jan. - Feb. 1999 *PF* (48).

Several changes were reviewed in preparing this proposal. The need to validate a “countable range” for compendial organisms was discussed; the Subcommittee decided that it would not be appropriate since countable ranges of between 25 and 250 have been deemed acceptable for most bacteria. The proposed incubation period in liquid media was lengthened from five to seven days. The definition of treatment groups removed from the previous version was restored to clarify the requirements. It was suggested to use a so-called “industry standard” of 50% recovery. The Subcommittee did not know of such an “industry standard,” and no supporting documentation was supplied with the comment from the field. Therefore, the recovery of 70% was retained, which is in agreement with the PDA *Technical Report on Bioburden Recoveries*. The description of preferred media for recovery of injured organisms is deleted since it was incomplete and appeared to confuse the readers. A number of correspondents requested that the statistical analysis included in the proposal be deleted, on the grounds that “microbiology is too variable.” The Subcommittee reviewed these comments and decided that because of the variability, statistical analysis is necessary to assess the results.

This proposed new informational chapter appeared in the *Tenth Supplement* (49), with an official date of May 15, 1999.

#### **<2021> Microbial Enumeration Tests - Nutritional and Dietary Articles**

Several general information chapters are under development to deal with nutritional supplements. The MCB Subcommittee’s main concern with these is in the microbial load the products may carry. The proposal for chapter <2021> arose from a number of requests for

some compendial guidance in this area. Appearing as a *Pharmacopeial Preview* in the Sept. - Oct. 1999 *PF* (50), this chapter and its companion chapter <2022> *Microbiological Procedures for Absence of Objectionable Microorganisms in Nutritional and Dietary Articles* follow the precedent set for the *Microbial Limits Test* of separating enumeration from identification tests. This proposal included four enumeration tests: Total Aerobic Microbial Count, Total Combined Molds and Yeasts Count, Coliform Count, and Enterobacterial Count. The Total Aerobic Microbial Count test includes membrane filtration, pour plate, or spread plate procedure. In the Coliform Count test, Lauryl Tryptose Broth and the most probable number determination are employed. The limits for the various enumeration tests are specified in the individual monographs; otherwise the guidance for establishing limits were provided under <2023> *Microbiological Attributes of Nonsterile Nutritional and Dietary Articles*, a new chapter appearing elsewhere in this issue of *PF*.

Each nonsterile nutritional or dietary article need not be tested by all four enumeration tests. It is expected that Total Aerobic Microbial Count and Total Combined Molds and Yeasts Count be performed on all nonsterile articles; in addition, depending on the article, either a Coliform Count or a Enterobacterial Count will be specified in the individual monograph.

#### **<2022> Microbiological Procedures for Absence of Objectionable Microorganisms in Nutritional and Dietary Articles**

This proposal for a new chapter appeared as a *Pharmacopeial Preview* in the Sept. - Oct. 1999 *PF* (51). It describes microbiological procedures for demonstrating the absence of objectionable microorganisms in raw materials, excipients, nutritional and dietary active substances, and nonsterile nutritional and dietary supplements. The procedures for determining the absence of *Staphylococcus aureus*, *Salmonella* species, *Escherichia coli*, and *Clostridium* species are described. However, these are not the only objectionable microorganisms possibly present in nonsterile pharmacopeial articles.

Objectionable microorganisms whose absence needs to be assessed will be specified in the individual monographs. A guidance for manufacturers in deciding which objectionable microorganisms have to be absent from the particular nutritional or dietary article is provided

under <2023> *Microbiological Attributes of Nonsterile Nutritional and Dietary Articles*, which also appeared in this issue of *PF*.

### <2023> **Microbiological Attributes of Nonsterile Nutritional and Dietary Articles.**

This new chapter mirrors <1111> *Microbiological Attributes of Nonsterile Pharmacopeial Articles* in that it provides guidance to the manufacturers in the testing of microbiological attributes. Appearing as a *Pharmacopeial Preview* in the Sept. - Oct. 1999 *PF* (52), it provided target values indicated for microbial enumeration tests for the nutritional and dietary articles depend on the origin of these articles, that is, whether these are botanical substances, botanical extracts, or articles of natural origin other than botanicals. Even though the absence of certain objectionable microorganisms may be established, the manufacturers would have to test any other objectionable organisms even if not specified in the monograph, when such organisms may pose a threat to the user.

### **Stimuli to the Revision Process**

The *Pharmacopeial Forum* provides a mechanism for interested parties in the field to publish scientific articles of interest to the audience. During the 1995 - 2000 revision cycle there were several articles on topics handled by the USP MCB.

### **Antimicrobial Efficacy and Sterility Testing**

*Predictive Antimicrobial Preservative Effectiveness Testing*. Samuel Shalkowsky. President, Spiral Biotech, Inc. *PF* 22 (4):2690 July - Aug. 1996.

This article describes a probability model to predict antimicrobial effectiveness from short-term experiments measuring inhibition of growth. This would allow for a test over a short period of time that would accurately predict the results of the compendial test. This rapid antimicrobial test could be used for preservative screening studies, or might even replace the compendial assay. In addition to the discussion of the probability model, the author also discusses several factors of the test and handling of the challenge organisms that would have a direct bearing on the final data.

*The Use of Preservatives In Compendial Articles.*

R. Dabbah, W-W Chang, and M. Cooper. USP Staff and USP MCB. *PF* 22 (4):2696 July - Aug. 1996.

This overview is based on an electronic search of USP 23 - NF 18, and the 1995 Physician's Desk Reference for monographs and prescription drugs containing preservatives. The results are presented in a series of tables. It is interesting to note that the number of different preservatives currently in use with prescription drugs is rather limited. When one takes into account the route of administration, this variety of preservatives used is even more restricted within a category of drug.

*Harmonization of Microbiological Methods - A Status Report*. Roger Dabbah, USP Division of Standards Development & Joseph E. Knapp, USP Microbiology Subcommittee Chairman. *PF* 23 (6):5334. Nov. - Dec. 1997.

This article retrospectively reviewed the harmonization of microbiological methods among the pharmacopeias of Europe, Japan, and the United States at a time when most of the contentious issues had been analyzed, discussed, and considered. International face-to-face meetings among the experts of the pharmacopeias as well as formal Open Conferences have resulted, in most cases, in advances in harmonization. The USP Open conference on Microbiology at Sanibel Island, Florida, and the Interpharmacopeial Open Conference in Barcelona, Spain, both held in 1996, as well as numerous meetings of the Pharmacopeial Discussion Group (PDG), were instrumental in defining, discussing, and resolving a number of harmonization issues.

*The Effects of Antimicrobial Preservatives On Organisms Derived from Fresh Versus Frozen Cultures*. Heidi Muth and Warren Casey. Glaxo Wellcome Research and Development *PF* 26 (2):519 March - April 2000.

Fresh and frozen cultures of *E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans*, *A. niger* and *B. cepacia* were exposed to various concentrations of sodium hypochlorite, benzalkonium chloride, and propyl parabens. The subsequent decrease in viability was measured by a turbidimetric method. No signifi-

cant differences were seen in this study in the response of fresh versus frozen cultures to challenge by the preservatives used. The authors conclude that frozen cultures are a justifiable alternative to fresh cultures for use in the antimicrobial preservative effectiveness test.

### Water Activity in Microbial Limits Testing

*The Application of Water Measurement to the Microbiological Attributes Testing of Nonsterile Over-The-Counter Drug Products.* R. R. Friedel, Whitehall-Robbins Healthcare R&D and A. M. Cundell, Wyeth-Ayerst Pharmaceuticals. *PF* **24** (2):6087. March - April 1998.

The authors discuss application of water activity ( $a_w$ ) measurements to the microbial limit testing of nonsterile pharmaceutical and over-the-counter (OTC) drug products. A knowledge of the formulation and water activity of a drug product may be used to establish an appropriate microbial monitoring program. Products manufactured in compliance with current good manufacturing practices that have low water activities will have little or no risk of microbial contamination. The authors contend that routine full microbial limit testing would not be indicated for these products.

*The Application Of Water Activity Measurement To Microbiological Attributes Testing Of Raw Materials Used In The Manufacture Of Nonsterile Pharmaceutical Products.* Robert R. Friedel, Perritt Laboratories. *PF* **25** (5):8974. Sept. - Oct. 1999.

The purpose of this study was to assess the potential of pharmaceutical raw materials to support growth of microorganisms according to their water activities. The authors chose raw materials commonly used in the formulation of nonsterile pharmaceuticals, over-the-counter drugs, and consumer products. Generally, chemicals with high water activities were shown far more likely to support growth of microorganisms. The authors conclude that the susceptibility of a raw material (actives and excipients) to microbial growth (and potential spoilage) should be a major factor in establishing adequate testing requirements for the material.

### Rapid Microbiological Methods

*Satisfying Microbiological Concerns for Pharmaceutical Purified Waters Using a Validated Rapid Test Method.* K. Wills, H. Woods, L. Gerdes, A. Hearn, N. Kyle, P. Meighan, N. Foote, K. Layte, and M. Easter; *Celsis-Lumac*, Cambridge Science Park, Cambridge, UK. *PF* **24** (1):5645 Jan. - Feb. 1998.

The authors describe an application of bioluminescence technology to perform a rapid check on the level of contamination in water. They note that trend analysis and timely response to out-of-specification results are recognized as the most desirable attributes of any monitoring procedure for water processes. However, conventional techniques for the detection of microorganisms are unsuitable because they rely on the detection of visible colonies, which can take up to seven days.

A rapid, quantitative, microbiological method is described, which gives results in one third of the time of conventional methods. It involves a novel combination of the recognized classical approaches (plate counting, growth in liquid media) and addresses many of the current microbiological concerns about validation of alternative methodologies.

The method has been validated and provides an effective tool for the microbial monitoring of process water. This facilitates timely system control and gives greater confidence for raw material control, with all the implications that it has for finished product safety and quality.

*Solid-Phase Laser-Scanning Cytometry: A New Two-Hour Method For The Enumeration Of Microorganisms In Pharmaceutical Water.* D.L. Jones, M. A. Brailsford, and J-L. Drocourt; Chemunex S.A., Paris, France. *PF* **25** (1):7626 Jan. - Feb. 1999.

The authors describe an alternate methodology to perform a rapid check on the level of contamination in water. The method described in this paper combines established techniques, including membrane filtration, labeling with a fluorescent viability substrate, and epifluorescence microscopy, with laser cytometry for the rapid enumeration of microorganisms in pharmaceutical process water.

This technology is the first to allow the labeling, detection, and direct counting of viable vegetative cells, bacterial spores, yeast, and mold with a single viability substrate. The protocol provides results within two hours, circumvents the restrictions imposed by cell growth, is highly sensitive, and has a very wide functional range. It allows the manufacturer to respond rapidly to an Out-of-Specification result (OOS) by providing an accurate, near real-time trend analysis.

This new method was validated using the USP guidelines in <1225> *Validation of Compendial Methods*, with modifications necessary for the validation of a microbiological test. Data from a multi-site study of pharmaceutical water systems (including Water for Injection and Purified Water) demonstrated the equivalence of this method to plate culture methods. The cytometer provides microbial counts in less than two hours that are equivalent to those obtained using a plate method optimized to maximally recover viable microorganisms in water (R2A, a low-nutrient medium incubated at 20 to 25°C for 14 days). It is more accurate with some water samples due to the limitations of classical cultivation methods.

## Conclusions

This has been a very busy revision cycle for the MCB Subcommittee. Table 1 provides a summary of the number of different publications for each chapter. It is clear from this table that even after a chapter is “finalized” and published in a *USP Supplement*, there is still opportunity to improve it or change aspects of the test. The USP is committed to continuous revision and improvement, and looks for input from the field.

The pace and approach used by the MCB Subcommittee has occasionally been characterized as too conservative, preventing practitioners from taking advantage of new technological advances in microbiological sciences.

The nature of referee tests in *USP* are such that these tests must be feasible in the laboratories of regulatory agencies as well as those of a variety of large and small manufacturers. Many of these new technologies are

expensive, and all are proprietary and thus cannot be included in *USP* as referee tests. It should also be noted that *USP* allows the use of alternate methods to *USP* referee methods provided that they are shown to be equivalent or better<sup>6</sup>. Given the special nature of microbiology, a separate informational chapter on validation of alternative methods is under development by the Subcommittee for future publication in *Pharmacoepial Forum*.

The new chapter on validation of alternative microbiological methods is not the only new chapter under development. Many comments come in to *USP* on new guidance that workers would like to see and many of these are under development. In addition, there are numerous existing chapters (<1211> Sterilization and Sterility Assurance chief among them) that are far past time for revision. The need for change has not ended with the 1995 - 2000 revision cycle. The next cycle promises to be every bit as active.

<sup>1</sup> “USP Open Conference on Microbiological Compendial Issues” held in January 1996 at Sanibel Harbour, Fort Myers, Florida.

<sup>2</sup> “Harmonization of the Sterility Test and the Antimicrobial Efficacy Test” was held in Barcelona, Spain in February 1996.

<sup>3</sup> “USP Open Conference on Microbiology in the 21<sup>st</sup> Century” held in New Orleans, LA, in May 1998.

<sup>4</sup> 1999 Meeting of the Pharmacopeial Discussion Group in Strasbourg, France, in September 1999.

<sup>5</sup> USP Open Conference on Water, Microbiology, and Particulates in Colorado Springs, CO, July 1995.

<sup>6</sup> “Compliance may be determined also by the use of alternative methods, chosen for advantages in accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction or in other special circumstances. Such alternative or automated procedures or methods shall be validated. However, Pharmacopeial Standards and procedures are interrelated; therefore, only the result obtained by the procedure given in this Pharmacopeia is conclusive.” *USP General Notices: Tests and Assays - Procedures*.

**Table 1: Summary of Activity.**

	Pharmacoepial Preview	In-Process Revision	Finalized	Total
<51> Antimicrobial Preservatives – Effectiveness		3 + 1*	1	5
<52> Antimicrobial Effectiveness Testing for Vaccines		1		1
<55> Biological Indicators – Resistance Performance Tests		1 + 1	1	3
<61> Microbial Enumeration Tests	1			1
<62> Microbial Procedures for Absence of Objectionable Microorganisms	1			1
<71> Sterility Tests		3 + 1	1	5
<85> Bacterial Endotoxins Tests		1		1
<1035> Biological Indicators for Sterilization		2		2
<1111> Microbiological Attributes of Nonsterile Pharmacoepial Articles	2			2
<1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments		3 + 1	1	5
<1207> Sterile Product Packaging – Integrity Evaluation	1			1
<1208> Sterility Testing – Validation of Isolator Systems	1	1		2
<1222> Terminally Sterilized Pharmaceutical Products - Parametric Release	1			1
<1227> Validation of Microbial Recovery from Pharmacoepial Articles	1	2	1	4
<2021> Microbial Enumeration Tests – Nutritional and Dietary Articles	1			1
<2022> Microbiological Procedures for Absence of Objectionable Microorganisms in Nutritional and Dietary Articles	1			1
<2023> Microbiological Attributes of Nonsterile Nutritional and Dietary Articles	1			1

\* This notation signifies the number of *In-process Revisions* prior to, and then following, publication of a finalized form of the chapter during the 1995 – 2000 revision cycle.

**Table 2: Stimuli Articles Published.**

<b>Stimuli Article</b>	<b>References</b>
<i>The Effects Of Antimicrobial Preservatives On Organisms Derived From Fresh Versus Frozen Cultures.</i> H. Muth, and W. Casey; Glaxo Wellcome Research and Development	<b>26</b> (2):519      Mar-Apr 2000
<i>The Application Of Water Activity Measurement To Microbiological Attributes Testing Of Raw Materials Used In The Manufacture Of Nonsterile Pharmaceutical Products.</i> Robert R. Friedel, Perritt Laboratories	<b>25</b> (5):8974.      Sept-Oct 1999
<i>Solid-Phase Laser-Scanning Cytometry: A New Two-Hour Method For The Enumeration Of Microorganisms In Pharmaceutical Water.</i> D.L. Jones, M. A. Brailsford, and J-L. Drocourt; Chemunex S.A., Paris, France.	<b>25</b> (1):7626      Jan-Feb 1999
<i>The Application Of Water Measurement To The Microbiological Attributes Testing Of Nonsterile Over-The-Counter Drug Products.</i> R. R. Friedel, Whitehall-Robbins Healthcare R&D and A. M. Cundell, Wyeth-Ayerst Pharmaceuticals	<b>24</b> (2):6087      Mar-Apr 1998
<i>Satisfying Microbiological Concerns For Pharmaceutical Purified Waters Using A Validated Rapid Test Method.</i> K. Wills, H. Woods, L. Gerdes, A. Hearn, N. Kyle, P. Meighan, N. Foote, K. Layte, and M. Easter; <i>Celsis-Lumac</i> , Cambridge Science Park, Cambridge, UK	<b>24</b> (1):5645      Jan-Feb 1998
<i>Harmonization Of Microbiological Methods – A Status Report.</i> Roger Dabbah, USP Division of Standards Development & Joseph E. Knapp, USP Microbiology Subcommittee Chairman.	<b>23</b> (6):5334.      Nov-Dec 1997
<i>Predictive Antimicrobial Preservative Effectiveness Testing.</i> Samuel Shalkowsky. President, Spiral Biotech, Inc.	<b>22</b> (4):2690      July-Aug 1996
<i>The Use Of Preservatives In Compendial Articles.</i> R. Dabbah, W-W Chang, and M. Cooper. USP Staff and USP MCB.	<b>22</b> (4):2696      July-Aug 1996

**Table 3: USP Conferences Held.**

<b>Conference Title</b>	<b>Date and Location</b>
USP Open Conference on Microbiological Compendial Issues	January, 1996 in Sanibel Harbour, Fort Myers, Florida
Harmonization of the Sterility Test and the Antimicrobial Efficacy Test	February, 1996 in Barcelona, Spain
North American Conference on Setting Specifications for Drug Substances and Drug Products” A joint conference of the FDA, USP, PDA, AAPS, and FIP on the ICH Q6A document.	September, 1996 in Washington, DC
USP Workshop on “Microbiology and Pharmaceutical Water”	April, 1997 in San Juan, Puerto Rico
USP Open Conference on Microbiology in the 21 <sup>st</sup> Century	May, 1988 in New Orleans, Louisiana

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1. *Pharmacopeial Forum* **18** (2): 3048 Mar-Apr 1992.
2. *Pharmacopeial Forum* **21** (4):1040 July-Aug 1995.
3. *Pharmacopeial Forum* **22** (6):3212 Nov-Dec 1996.
4. *Pharmacopeial Forum* **23** (2):3806 Mar – Apr 1997.
5. *Pharmacopeial Forum* **23** (2):3806 Mar – April 1997.
6. *Eighth Supplement to The United States Pharmacopeia 23 / National Formulary 18*. 1994. The United States Pharmacopeial Convention, Inc. Rockville, MD. p. 1681 effective May 15, 1998.
7. *Pharmacopeial Forum* **23** (5):5334 Nov-Dec 1997.
8. *Pharmacopeial Forum* **25** (1):7513 Jan–Feb 1999.
9. *Pharmacopeial Forum* **24** (3):6269 May-June 1998.
10. *Pharmacopeial Forum* **20** (6):8439, Nov–Dec 1994.
11. *Pharmacopeial Forum* **22** (5):2862 Sept – Oct 1996.
12. *Sixth Supplement to The United States Pharmacopeia 23 / National Formulary 18*. 1994. The United States Pharmacopeial Convention, Inc. Rockville, MD. p. 3754, effective May 15, 1997.
13. *Pharmacopeial Forum* **25** (3):8223 May-June 1999.
14. *Pharmacopeial Forum* **25** (2):7761 Mar-Apr 1999.
15. *Pharmacopeial Forum* **25** (2):7774 Mar-Apr 1999.
16. *The United States Pharmacopeia 23 / National Formulary 18*. 1994. The United States Pharmacopeial Convention, Inc. Rockville, MD. p. 1686.
17. May-June 1995 issue of *Pharmacopeial Forum* (21(3):595 May-June 1995).
18. *Pharmacopeial Forum* **21** (5):1250 Sept-Oct 1995.
19. *Pharmacopeial Forum* **22** (5):2868 Sept-Oct 1996.
20. *The United States Pharmacopeia 23 / National Formulary 18*. 1994. The United States Pharmacopeial Convention, Inc. Rockville, MD. p. 10.
21. *Pharmacopeial Forum* **23** (6):5182 Nov-Dec 1997.
22. *Pharmacopeial Forum* **23** (6):5334 Nov-Dec 1997.
23. *Eighth Supplement to The United States Pharmacopeia 23 / National Formulary 18*. 1994. The United States Pharmacopeial Convention, Inc. Rockville, MD. p. 4295, effective May 15, 1998.
24. *Pharmacopeial Forum* **24** (5):6926 Sept-Oct 1998.
25. *European Pharmacopoeia – Supplement 2001* 2.6.1 Sterility.
26. *Tenth Supplement to The United States Pharmacopeia 23 / National Formulary 18*. 1994. The United States Pharmacopeial Convention, Inc. Rockville, MD. p. 4923, effective May 15, 1999.
27. *Pharmacopeial Forum* **26** (4):1102 July-Aug 2000.
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29. *Pharmacopeial Forum* **26** (1):219 Jan-Feb 2000.
30. *Pharmacopeial Forum* **20** (6):8461 Nov-Dec 1994.
31. <1035> Biological Indicators The United States Pharmacopeia 23 / National Formulary 18. 1994. The United States Pharmacopeial Convention, Inc. Rockville, MD. p. 1847.
32. *Pharmacopeial Forum* **23** (4):4520 July-Aug 1997.
33. <1111> Microbiological Attributes of Nonsterile Pharmaceutical Products. *The United States Pharmacopeia 23 / National Formulary 18*. 1994. The United States Pharmacopeial Convention, Inc. Rockville, MD. p. 1939.
34. *Pharmacopeial Forum* **18** (4):3596 July-Aug 1992.

35. *Pharmacopeial Forum* **22** (6):3098 Nov-Dec 1996.
36. *Pharmacopeial Forum* **25** (2):7785 Mar-Apr 1999.
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43. *Pharmacopeial Forum* **23** (6):5068 Nov-Dec 1997.
44. *Pharmacopeial Forum* **25** (1):7562 Jan-Feb 1999.
45. *Pharmacopeial Forum* **23** (6):5074 Nov-Dec 1997.
46. *Pharmacopeial Forum* **22** (6):3109 Nov-Dec 1996.
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49. *Tenth Supplement to The United States Pharmacopeia 23/National Formulary 18*. 1994. The United States Pharmacopeial Convention, Inc. Rockville, MD. p. 5063, effective May 15, 1999.
50. *Pharmacopeial Forum* **25** (5):8748 Sept-Oct 1999.
51. *Pharmacopeial Forum* **25** (5):8761 Sept-Oct 1999.
52. *Pharmacopeial Forum* **25** (5):8770 Sept-Oct 1999.