

Activities of the USP Analytical Microbiology Expert Committee During the 2000–2005 Revision Cycle

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ABSTRACT: This article is a comprehensive review of the published activities of the Analytical Microbiology Expert Committee (AMB) for the 2000–2005 revision cycle. The major thrust of the activities during this revision cycle were directed at international harmonization, and to provide guidance in the changing field of pharmaceutical microbiology. In addition to reviewing the changes accomplished, this article discusses the rationale for many of the changes and some background information regarding new initiatives underway. Where appropriate, changes in the USP that did not fall under the direct purview of the AMB Expert Committee (EC) but of interest to the microbiology community are also discussed.

Introduction

The five-year period of 2000–2005 has continued a period 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 increased role of USP in describing laboratory methods, microbiology-related process controls, and introduction of rapid microbiological methods have all been discussed by the committee in published drafts. In addition to the creation of new guidance chapters, there has been a strong drive to update older chapters and towards international harmonization of referee chapters and critical monographs. Many of the publications that occurred in 2000 are actually the work of the Subcommittee during the 1995–2000 revision cycle (1) and these are included in this review with the acknowledgement of the hard work of the previous Subcommittee, many of whose members continue to serve. (Prior to the 2000–2005 cycle of revision, Expert Committees were referred to as Subcommittees of the USP Committee of Revision.)

The Analytical Microbiology Committee of Experts of the United States Pharmacopeial Convention is re-

sponsible for both general and informational chapters dealing with microbial assays and microbial control of processes. The committee's responsibility does not extend to drug or product monographs, which are handled by other Committees, although the AMB does support the development of microbial requirements for all monographs through consultation with the relevant monograph committee. Similarly, antibiotics are addressed by a separate Expert Committee, as are the monographs and informational chapter on waters.

The AMB Expert Committee develops or revises chapters as the need arises. There are two distinct types of chapters encompassed by USP: standards and guidance documents. USP standards, microbiological methods included, include referee tests that have a scientific as well as a legal standing; they are expert standards, not consensus standards. This is an important point as the review process solicits information from academia, industry and the government, but it is the members of the committee who make the final determination. As reference tests, they are geared towards support of product monographs as described in the *NF*. 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 "mis-labeled" 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 information chap-

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ters in USP are widely held as reference guides for industry, and changes to the official chapters are very slowly introduced.

The revision process of the *USP* is designed to provide the maximum opportunity for input into the deliberative process. Interested parties, which may also include members of the committee, propose a new chapter or changes to current chapters; these proposals are then forwarded to the Expert Committee 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 may appear as a *Pharmacopeial Preview*; otherwise, the proposal is published in the *In-Process Revision* section of *PF*.

After it receives public comments, the AMB committee of experts reviews 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 committee is satisfied that the proposal is ready to become official. At that point, the Expert Committee proposes the chapter for final adoption. If approved by balloting by the appropriate individuals, the chapter appears in a supplement or in the annual edition of the USP-NF. Supplements are the mechanism used to update the USP between the annual publications of the book.

If a significant number of comments are then received regarding the revised official chapter, it will be re-evaluated, and possibly a new round of *In-process Revision* drafts will be published and considered. Thus the revision process of USP is a continuous one, responsive to changing regulatory and industrial needs. A summary of the activity is provided in Table I. 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 the USP as approval.

A final method for introduction of changes into the pharmacopeia is through the inter-pharmacopeial harmonization process. This process is a slow and laborious method of reaching consensus among the regional pharmacopeia (USP, Pharm. Eur., and JP) in terms of the details of specific tests. The procedure for

this harmonization process is subject to change, and the interested reader should consult the current USP chapter “(1196) Pharmacopeial Harmonization” for the specifics of the process. Several microbiological chapters are currently the subject of the Harmonization process, including “(71) Sterility Tests”, “(61) Microbial Limits Tests” and “(1111) Microbial Attributes of Nonsterile Pharmacopeial Articles.” Proposed drafts of harmonized chapters are also published in the *Pharmacopeial Forum* in a separate section.

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 2000–2005 revision cycle there were several articles published of particular interest to the work of the Analytical Microbiology Expert Committee. These *Stimuli* articles are designed to promote discussion of new ideas or provide data to assist the Expert Committee in improving chapters in the *USP* and have been used in the revision process by the AMB Expert Committee. A listing of the relevant articles is provided in Table II, and each is discussed later in this article.

The *Pharmacopeial Forum* has become an important publication for the pharmaceutical microbiologist both for review of upcoming standards and for publication of scientific work. This can be seen in Figure 1 as the number of microbiology-related publications each year has shown a steady increase. In fact, if you examine the output of each of the last four committees (1985–1989, 1990–1994, 1995–1999 and 2000–2004) there is a linear increase in number of new chapters and revisions (Figure 2). While no one expects this trend to continue indefinitely, it does serve to underscore the increasing importance of microbiology in the pharmaceutical industry and the central role of the AMB EC.

To facilitate the ongoing discussion necessary for the revision process, meetings and conferences focusing on specific microbiological topics were held. These were well attended and generated discussion on proposals made or to be made by the AMB EC (see Table III).

This review will examine the published activity by chapter in numerical order with the exception of the Microbial Limits chapters which will be considered together. This number sequence is important in its own

TABLE I
Summary of Activity

	Pharmacopeial Preview	In-Process Revision	Finalized	Total
<51> Antimicrobial Preservatives—Effectiveness			1	1
<55> Biological Indicators—Resistance Performance Tests		2		2
<61> Microbial Enumeration Tests		2		2
<62> Microbial Procedures for Absence of Objectionable Microorganisms		1		1
<71> Sterility Tests		1	1	2
<85> Bacterial Endotoxins Tests			1	1
<1035> Biological Indicators for Sterilization		1	1	2
<1072> Disinfectants and Antiseptics	1	2		3
<1111> Microbiological Attributes of Nonsterile Pharmacopeial Articles		2		2
<1112> Application of Water Activity Determination to Nonsterile Pharmaceutical Products	1	1		2
<1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments		1		1
<1117> Microbiological Best Laboratory Practices	1	1		2
<1207> Sterile Product Packaging—Integrity Evaluation		1	1	2
<1208> Sterility Testing—Validation of Isolator Systems		2		2
<1209> Sterilization—Chemical and Physicochemical Indicators and Integrators		1	1	2
<1211> Sterilization and Sterility Assurance of Compendial Articles		1		1
<1222> Terminally Sterilized Pharmaceutical Products—Parametric Release		2	1	3
<1223> Validation of Alternative Microbiological Methods	1	1		2
<1227> Validation of Microbial Recovery from Pharmacopeial Articles				0
<2021> Microbial Enumeration Tests—Nutritional and Dietary Articles		1		1
<2022> Microbiological Procedures for Absence of Specified Microorganisms in Nutritional and Dietary Supplements	0	1		1
<2023> Microbiological Attributes of Nonsterile Nutritional and Dietary Articles		1	1	2

right, as there is an underlying structure to the numbering sequence of USP Chapters. Chapters numbered less than <1000> contain referee tests that are enforceable by regulatory agencies. Those chapters numbered from <1000> to <1999> are information chapters. Those

chapters beginning at <2000> are devoted to nutritional supplements and may be enforceable should the manufacturer elect to claim compliance with USP standards. Otherwise, those chapters are informational. Chapters reviewed in this article include:

TABLE II
Stimuli Articles Published

Stimuli Article	Reference
<i>The FDA Process Analytical Technology (PAT) Initiative—an Alternative Pharmaceutical Manufacturing Practice (aPMP)</i> USP Project Team 18—Process Analytical Technology	30 (6):2254–2264 Nov–Dec 2004
<i>The USP Perspective to Minimize the Potential Risk of TSE Infectivity in Bovine-Derived Articles Used in the Manufacture of Medical Products.</i> Deveau, I, <i>et al</i>	30 (5):1911–1921 Sept–Oct 2004
<i>Sterilizing Filtration with Microporous Membranes.</i> Jornitz, MW and TH Meltzer	30 (5):1903–1910 Sept–Oct 2004
<i>Microbial Identification in the Pharmaceutical Industry.</i> Sutton, SVW and AM Cundell	30 (5):1884–1894 Sept–Oct 2004
<i>Significant Digits and Rounding</i> Torbeck, L	30 (3):1090–1095 May–June 2004
<i>The Role of Rapid Microbiological Methods Within the Process Analytical Technology Initiative.</i> Singer, DC and AM Cundell	29 (6):2109–2113 Nov–Dec 2003
<i>Review of the Media Section and Incubation Conditions For the Compendial Sterility and Microbial Limit Tests.</i> Cundell, AM	28 (6):2034–2041 Nov–Dec 2002
<i>Comparison of Microbiological Testing Practices In Clinical, Food, Water and Pharmaceutical Microbiology In Relation to the Microbiological Attributes of Nutritional and Dietary Supplements.</i> Cundell, AM	28 (3):964–985 May–Jun 2002
<i>An Alternative Methodology for the General Test Chapter Microbial Limit Tests (61).</i> Casey, W <i>et al</i>	27 (1):2046–2050 Jan–Feb 2001
<i>The Effects Of Antimicrobial Preservatives On Organisms Derived From Fresh Versus Frozen Cultures.</i> H. Muth, and W. Casey	26 (2):519 Mar–Apr 2000

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| <51> Antimicrobial Effectiveness Testing | <1111> Microbiological Attributes of Nonsterile Pharmaceutical Products |
| <55> Biological Indicators–Resistance Performance Tests | <1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments |
| <61> Microbial Limit Tests (Microbial Enumeration Tests) | <1117> Microbiological Best Laboratory Practices |
| <62> Microbiological Procedures for Absence of Objectionable Microorganisms | <1207> Sterile Product Packaging—Integrity Evaluation |
| <71> Sterility Tests | <1208> Sterility Testing—Validation of Isolator Systems |
| <85> Bacterial Endotoxins Test | <1209> Sterilization—Chemical and Physicochemical Indicators and Integrators |
| <1035> Biological Indicators for Sterilization | |
| <1072> Disinfectants and Antiseptics | |

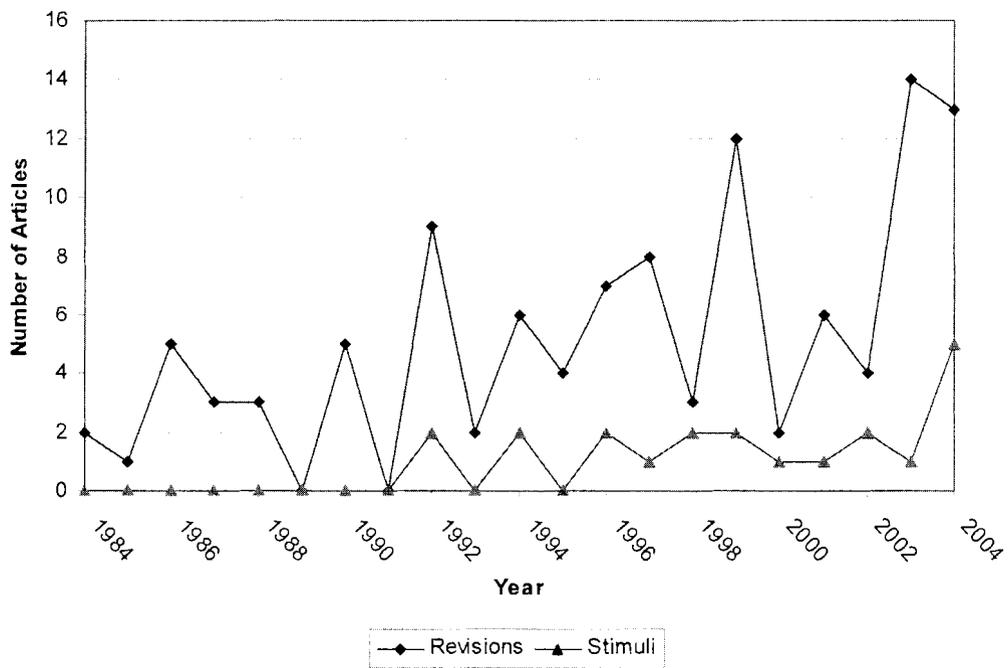


Figure 1

Microbiology—Related Publications in Pharmacopeial Forum: 1984–2004

<1211> Sterilization and Sterility Assurance of Compendial Articles

<1223> Validation of Alternative Microbiological Methods

<1222> Terminally Sterilized Pharmaceutical Products—Parametric Release

<1227> Validation of Microbial Recovery from Pharmacopeial Articles

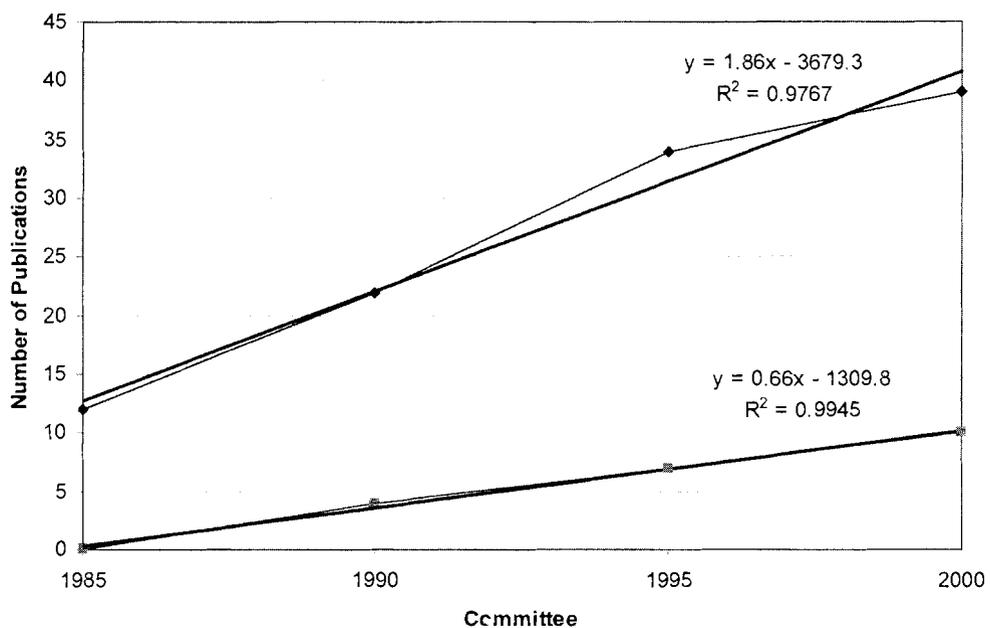


Figure 2

Pharmacopeial Forum publications of Recent Committee of Revisions

TABLE III
USP Conferences Held

Conference Title	Date and Location
“Sterile Product Manufacturing” a joint conference of USP and PDA	May 19–22, 2002 in Sanibel Harbour, Florida
USP Biotechnology Conference	November 18–21, 2003 in Washington, DC
USP First Annual Scientific Meeting	September 25–28, 2004 in Iselin, NJ

⟨1231⟩ Water for Pharmaceutical Purposes

⟨2021⟩ Microbial Enumeration Tests—Nutritional and Dietary Supplements

⟨2022⟩ Microbiological Procedures for Absence of Specified Microorganisms—Nutritional and Dietary Supplements.

⟨2023⟩ Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements.

⟨51⟩ Antimicrobial Preservatives—Effectiveness

There were no revisions proposed to this chapter after a period of intense activity in the past revision cycle aside from the elimination of the nonaqueous category and the addition of the category for liquid antacids made with an aqueous base which appeared in USP 25. Two reviews of the status of this chapter were, however, published in the peer reviewed literature. The first was written from the perspective of the USP (2) and the other from the European (3). A proposed new chapter, “⟨52⟩ Antimicrobial Effectiveness Testing for Vaccines” was officially cancelled in 2001 as the effort to harmonize the Antimicrobial Effectiveness Test was abandoned (4).

⟨55⟩ Biological Indicators—Resistance Performance Tests

The July–August 2001 *PF* contained an in-process revision to General Chapter ⟨51⟩ that included an expanded discussion of the appropriate use of the Limited Spearman-Kärber Method for determining the D-value (5). In addition, the equation for the calculation of V_7 , the variance of the mean heating time for achieving complete kill, was corrected. Several editorial changes were also proposed. These changes became official in USP 26 (2003)

Further changes were proposed to this chapter in the Jan-Feb 2004 *PF* where it was proposed to revise the

sections on Total Viable Spore Count and D-Value Determination (6). It was further suggested to add requirements specified in the several newly proposed monographs for Biological Indicators of different types that appeared in the same issue (Moist Heat, Dry Heat and Gaseous Modes of Sterilization, and Liquid Spore Suspensions).

⟨61⟩ Microbial Limit Tests (Microbial Enumeration Tests)

The harmonization of the Microbial Limits Tests, which had begun in the 1995–2000 revision cycle, continued throughout the 2000–2005 revision cycle. A discussion of the international harmonization process, and a listing of USP monographs and general chapters undergoing the publication of the proposed harmonized documents for USP chapters ⟨61⟩, ⟨62⟩ and ⟨1111⟩ each appeared as *Pharmacopeial Previews* in 1999 (6, 7, 8). The proposals included splitting the existent USP general chapter “⟨61⟩ Microbial Limits Tests” into two chapters, one dealing with microbial enumeration (bioburden) and the other the absence of “objectionable” microorganisms.

The official Stage 4 harmonization document for chapter ⟨61⟩ was published in *PF* of March-April 2001 (10). The harmonization proposal contained significant changes from the previous version based on discussions held in the Pharmacopoeial Discussion Group meeting in July of 2000. These changes included:

- Changing the title of the chapter from “Microbial Enumeration Tests” to “Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests.”
- Deletion of references to coliform bacteria and enterobacteria
- Evaluation of growth promotion and bacteriostasis/fungistasis to require that the test material not

differ from the controls by more than 0.3 log₁₀ units.

- Addition of a procedure for testing Transdermal patches
- The use of a single filter, rather than duplicates, for sample enumeration
- The alteration of several incubation periods:
 - 2–3 days for *C. albicans*
 - 5–7 days for *A. niger*
 - Not more than 2 days for bacteria and not more than 4 days for yeasts and molds for bacteriostasis/fungistasis testing.

This proposal generated a great deal of discussion, and the AMB committee, in consultation with the European Pharmacopoeia and the Japanese Pharmacopoeia, made extensive revisions in the proposed Stage 4 document. Therefore, the initial Stage 4 document was canceled and re-released in the Sept–Oct 2003 issue of *Pharmacopoeial Forum* (11). Changes to the document included:

- References to method “validation” were replaced by the more appropriate terminology “verification of suitability of the method”.
- The section describing preparation of test strains was modified in order to allow more flexibility (e.g., use of commercial strains instead of freshly prepared stock; the use of solid as well as liquid agar to grow bacterial strains; the use of Sabouraud-Dextrose Medium for *Candida albicans*; the use of Potato Dextrose Agar Medium for growth of *Aspergillus niger*; the use of stable spore suspensions; and storage of the test organism suspension for up to 24 hours.)
- The sections on growth promotion were significantly expanded (e.g., which microorganism grows on which medium, incubation conditions). Criteria for liquid media were added. In the tests for specified microorganisms, a new section for verification of the selectivity of the media was introduced: the verification of the suitability of the medium was clarified to be carried out with separate microorganism suspensions.

- More detailed instructions on how to cope with products containing antimicrobial agents were added throughout the texts. In particular, growth was considered inhibited if there was a reduction by a factor of 2 (0.3 log₁₀ units).
- Confidence limits for the most-probable-number (MPN) method were added.
- Regarding interpretation of the results, clarification was made with regard to the counting of colonies.
- Other changes of lesser significance were made throughout the text for the sake of grammatical or editorial requirements.

⟨62⟩ Microbiological Procedures for Absence of Objectionable Microorganisms

The 2001 publication of the Stage 4 harmonized chapter ⟨62⟩ (12) continued the 1999 harmonized proposal to separate the Microbial Limits Tests into two chapters, a change which by this time was completely accepted although no official change had yet occurred to the chapter (a state of affairs that continues to date). This version was also presented as the Stage 4 harmonized version, and contained major changes from the previous version:

- The title was changed from “⟨62⟩ Microbiological Procedures for Absence of Objectionable Microorganisms” to “⟨62⟩ Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms”
- The alternate use of Rappaport Vassiliadis Salmonella Enrichment Broth and Deoxycholate Citrate Agar for *Salmonella* testing was added.
- The alternate use of Buffered Sodium Chloride-Peptone Solution, pH 7.0 as a diluent was added.
- Fluid Lactose Medium for pre-incubation was deleted.
- Bile-tolerant gram-negative bacteria methods replaced the enterobacteria test
- The incubation period for Stage 2A was changed to a period of 18 to 48 hours.

This version was a dramatic change in philosophy from the previously proposed version. Rather than attempt to provide methods to demonstrate the absence of “objectionable” microorganisms (a description that would apply to different microorganisms in different formulations, and different microorganisms in different product presentations and patient populations) this version focused on specified microorganisms. The purpose of the Microbial Limits Tests has never been to provide methods to evaluate the absence of all potentially harmful microorganisms, but rather to provide test methods to evaluate the requirements for absence of specified organisms as required in the monographs (13).

This proposed new chapter underwent extensive changes in discussions of the PDG from 2001–2003 which required that the previous Stage 4 document be cancelled and replaced by a new PDG Stage 4 OFFICIAL INQUIRY in 2003 (14). The following changes were based upon comments received in response to the previous Stage 4 document and upon discussion held by experts from the EP, JP, and USP:

- Incubation temperature for *Escherichia coli* was raised to between 42°C and 44°C to provide better selectivity.
- Sample size was decreased to 1 g.
- Regarding selective agars for the detection of *Salmonella* species, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, only one medium was retained in each case.
- The method for growing *Candida albicans* has been replaced by the method used for the determination of TCYM.
- Specific instructions for the sterilization of media have been removed.
- Specific instructions for growth promotion of the media were added, including demonstration of the adequacy of the medium’s selective and differential properties.

⟨1111⟩ Microbiological Attributes of Nonsterile Pharmaceutical Products

The discussion of proposed revisions to USP chapter ⟨1111⟩ Microbiological Attributes of Nonsterile Phar-

maceutical Products” is included here as the three chapters (⟨61⟩, ⟨62⟩ and ⟨1111⟩) are inter-dependent. The 1999 proposal for this chapter revision (a Stage 3 document, as the development of a harmonized version of this chapter lagged behind the harmonization proposals for chapters ⟨61⟩ and ⟨62⟩) supported the position that the USP should be providing guidance on demonstration of the absence of objectionable microorganisms. The proposed chapter as published in 2002 (15) incorporated several major changes:

- The title was changed from “⟨1111⟩ Microbiological Attributes of Non-Sterile Pharmacopeial Articles” to ⟨1111⟩ Microbial Contamination Limits for Non-Sterile Products.”
- The section on “Formulation and process design” was deleted.
- The section on “Facilities, equipment, and water” was deleted.
- The section on “Drug product components” was deleted.
- The section on “Sanitization” was deleted.
- The section on “Sampling” was deleted.
- The table “Microbial Enumeration Limits for Raw Materials, Excipients and Drug Substances” was deleted.
- The decision tree for determining which objectionable organisms may be present was deleted.
- A decision tree was added for tests for specified microorganisms on selective media.
- A table was added showing microbial enumeration limits for herbal medicinal products.

This proposal was based on the desire of the PDG to avoid GMP issues, and to simplify the procedure as much as possible. However, while the proposed USP chapter ⟨62⟩ was moving away from incorporation of guidance on “objectionable microorganisms,” chapter ⟨1111⟩ continued to embrace this position. However, by 2003 this position had shifted significantly and was now in line with the rest of the microbial limits chapters (16). This 2003 proposal was presented as a Stage 4 document with the Stage 4 proposals for chapter ⟨61⟩

and chapter (62) and incorporated the following changes:

- The term “objectionable” was been deleted.
- The decision tree (Figure 1) for selection of objectionable microorganisms present on selective media was deleted because it caused confusion and the guidance it provided did not prove to be useful.
- The term “Microbial Enumeration Targets” was replaced by the term “Criteria for Microbiological Quality”.
- Criteria for total combined yeasts and molds count (TCYM) was added for preparations for oromucosal, gingival, cutaneous, nasal, auricular, vaginal, and inhalation uses, and for transdermal patches.
- Preparations for oral use were split into two categories (liquid and solid), because different acceptance criteria are applied in view of the different potential for contamination.
- Herbal drugs were excluded from the scope of International Harmonization, because they are dealt with differently from region to region.
- Nonsterile substances for pharmaceutical use were included into the scope of International Harmonization in order to give general guidance on microbiological quality. Specific monographs will continue to give mandatory requirements where appropriate.
- Other changes of lesser significance have been made throughout the text.

(71) Sterility Tests

The finalized and harmonized revision to USP chapter “(71) Sterility Tests” was published in 2000 (17) as an “Official Inquiry Stage 4” Harmonization Document. It should be noted that this harmonization effort had already resulted in significant changes to the USP Sterility Tests which had been implemented in the previous revision cycle (1). It was the result of a number of meetings of the PDG and representative experts. The text at this point was identical among the pharmacopeia, with the following exceptions:

- To take into account differing regulatory and control situations in among various regions, the European Pharmacopoeia (EP) text used a 7-day incubation period for steam-sterilized products, subject to authorization by the qualified regulatory authority.
- The USP text defined an Alternative Thioglycollate Medium that was to be used for medical devices that are labeled “Sterile Path”. The EP text did not define such a medium, since it did not include sterility testing of medical devices.
- In the USP text, but not in the EP text, special instructions were given for media that include beta-lactamase and were to be used for penicillin or cephalosporin products.
- For prepared media not used immediately, which were to be stored in tight containers at 2°C to 25°C, the EP indicated the use of “sealed” containers, whereas the USP indicated “tight” containers. Storage for not more than 6 months was indicated in the EP, with longer storage periods allowed if validated. The USP allowed storage for one year if the medium was tested for growth promotion within 3 months of the time of use. The USP also discussed Ready-to-Use Media, while the EP and the Japanese Pharmacopoeia (JP) did not.
- Microorganisms that were used for growth promotion and validation tests had the same ATCC numbers in the EP, the JP, and the USP. The EP also provided a listing of additional strains from other culture collections (CIP, NCIMB, NCPF, IP, and IML), and the USP provided alternatives to the ATCC species listed.
- The current USP Validation Tests for Bacteriostasis and Fungistasis were modified: Fluid Thioglycollate Medium was inoculated with one aerobic and one anaerobic microorganism, and Soybean-Casein Digest Medium was inoculated with one aerobic microorganism and either a mold or a yeast. Incubation at the appropriate temperature was changed from 7 to 5 days.
- Table 3 (*Quantities of Article for Liquid Products*) and Table 4 (*Quantities of Article for Solid Products*) were replaced by a single, simpler table.

- Sample preparations for the Membrane Filtration Method and the Direct Transfer Method were the same as in the EP and the JP, except that the USP provided additional instructions for Prefilled Syringes; Antibiotic Solids, Bulks and Blends; Purified Cotton, Gauze, Surgical Dressings, Sutures, and Related Articles; and Sterile Devices.

The finalized version underwent some minor modifications among which was the extension of the incubation period to 14 days for all products, and the inclusion of a 4 day incubation of subcultured sample after the 14 day incubation if the product rendered the media turbid. This document, with remaining regional differences, was then finalized amongst all three regional pharmacopeia early in 2003. The USP published the finalized version in the Fourth Interim Announcement (18) that took effect January 1, 2004.

⟨85⟩ Bacterial Endotoxins Test

The AMB has completed the harmonization initiative on BET with the EP and the JP. Final agreement on the harmonized document was completed in September of 1999 and the result was published in the Jan–Feb 2000 *PF* (19). It was included 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 for Sterilization

A proposed revision to USP guidance chapter “⟨1035⟩ Biological Indicators” was published in 2001 (20). The purpose of this revision was to clarify several sections. The definition of biological indicators was expanded, as were the description of their uses, and the discussion of the types of biological indicators (self-contained indicators in particular). More detail was provided for the preparation of biological indicators. A discussion of the “overkill method,” the 12 D process, and an updated section on *Vapor-Phase Hydrogen Peroxide* were provided in the section entitled “*Selection for Specific Sterilization Processes*.” In addition, clarification of the requirements for achieving the sterility assurance level of 10^{-6} by employing biological

indicators was added in the section “*Use for In-process Validation*.” These changes were incorporated into USP 25 (21).

⟨1072⟩ Disinfectants and Antiseptics

This proposed information chapter first appeared in the Jan/Feb *PF* of 2002 (22) in response to requests from industry to provide some information on the use of disinfectants, sanitizing agents and antiseptics in the manufacturing arena and the laboratory. There was significant confusion in the field on the terminology and application of these agents, as well as concern over regulatory expectations. The AMB EC could not directly address regulatory expectations, but it was felt that a significant contribution might be made in providing a base-line lexicon for the discussion. This chapter, therefore, provided a set of definitions heavily reliant on the standard text edited by Seymour Block (23). The chapter then presents a classification scheme for the agents based on their chemical nature, with proposed uses (germicide, antiviral, sporicide, etc). Following sections included guidance to help with the selection of an antiseptic for hand and surgical site selection, the selection of a disinfectant for in a pharmaceutical manufacturing environment, a theoretical discussion of disinfectant activity, a discussion of the mechanism of disinfectant activity and a short section on microbial resistance to disinfectants. Guidance was also provided in support of disinfectant challenge testing and the role of disinfectants in a cleaning and sanitization program.

A second proposed version to chapter “⟨1072⟩ Disinfectants and Antiseptics” was presented as an In-process Revision in 2003 (24). The scope of the chapter was clarified and a number of definitions were expanded and clarified. Modifications to the sections on “Microbial Resistance to Disinfectants and Disinfectant Challenge Testing” were made. Substantial changes were made to the section on “Disinfectants in a Cleaning and Sanitization Program” in an effort to clarify the concept of disinfectant rotation.

A third version of this chapter was presented in 2004 as an In-process revision (25). Further editorial changes were made in the text to improve clarity based on comments received from the field. In addition, the Table entitled *Biocidal Activity, Organic Inactivation, Residual Activity and Application of Some Common Disinfectants* was deleted due to concern over misapplication of the table. Specifically the concern was that

many of the applications described were very dependent on the concentration of the biocide used, and that concentration was not included in the table, nor could the table be readily modified to more accurately reflect this concern.

<1112> Application of Water Activity Determination to Nonsterile Pharmaceutical Products

The proposed USP guidance chapter first appeared as a *Pharmacopeial Preview* under the title “<1112> Microbiological Attributes of Nonsterile Pharmaceutical Products—Application of Water Activity Determination.” (26) This new general information chapter was designed to provide guidance on the influence of water activity as it pertains to product formulation susceptibility to microbial contamination. The chapter discussed the potential for improving product preservation by maintaining low water activity. A table was provided with data on water activity requirements for the growth of a range of microorganisms. Another table gave strategies for microbiological testing based upon product water activities. In addition, a method for the measurement of water activity was presented.

A revision of this chapter was published in the Sept–Oct 2004 PF based upon input from the field and further discussions within the AMB EC (27). The chapter progressed to *In-process Revision* status with changes:

- The title of the chapter was changed for clarity.
- The importance of container-closure integrity in maintaining the water activity level during the product shelf life was emphasized.
- It was clarified and reinforced that reduced microbial limits testing must be justified through risk assessment and not solely on water activity determinations.
- The ability of more resistant microorganisms to persist in drug products of low water activity was acknowledged.

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

Although this chapter was very controversial during the last revision cycle (1), there was no activity on it until late in 2000–2005 revision cycle. This could

have been because it was during this time that the FDA was publicly developing its long-awaited revision to the 1987 Aseptic Processing Guideline and all attention was focused on that effort (28). A completely revised proposal for this informational chapter was published early in 2005 (29).

<1117> Microbiological Best Laboratory Practices

The new USP chapter “<1117> Microbiological Good Laboratory Practices” first appeared as a *Pharmacopeial Preview* in the May–June 2003 issue of PF (30). This proposed chapter was developed in response to requests from the field to provide information to the microbiology laboratory. The chapter was organized into major topics of importance to the successful operation of a microbiology laboratory:

- **Media preparation and quality control**
This section useful information on different methods of media sterilization, and guidance on ways to perform appropriate growth promotion testing on lots of media. Guidance is also provided on media storage conditions.
- **The maintenance of microbiological cultures**
This section provides detailed guidance on “seed-lot culture” techniques.
- **The maintenance of laboratory equipment**
- **Laboratory layout and operations**
- **Training of personnel**
The need for appropriately trained technicians and supervisors was emphasized in this section. Microbiology is a specialized science and adequate supervision of the function demands managers who understand the topic.
- **Documentation**
- **The maintenance of laboratory records**
- **Interpretation of laboratory results**
This section is an attempt to provide guidance to investigations of “failures” or “data deviations” in the microbiology laboratory. This is a topic of some difficulty due to the variability inherent in compendial microbiological assays and the critical nature of most microbiology assays in terms of product lot disposition for a manufacturing facility.

The initial draft of this chapter received a great deal of commentary, most of it positive. The AMB committee presented a revision of this chapter in 2004 as an *In-process Revision* (31). In addition to numerous editorial changes, the sections on *Media Preparation and Quality Control*, *Maintenance of Microbiological Cultures*, and *Interpretation of Laboratory Results* underwent substantial revision. In response to specific request, the phrase “Good Laboratory Practices” was amended in the title. This was re-worded to “Best Laboratory Practices” to avoid confusion with clinical guidelines.

⟨1207⟩ Sterile Product Packaging—Integrity Evaluation

The proposed guidance chapter “⟨1207⟩ Sterile Product Packaging—Integrity Evaluation” appeared as an *In-process Revision* in the Sept–Oct *PF* of 2001 (32). It was a revision of the *Pharmacopeial Preview* version that had been published four years earlier (33). The new version included clarifications of the use of physical testing during product package development and the use of microbiological testing during routine manufacturing. In addition, the general discussion of the use of physical testing methods for package integrity assessment was clarified. The section *Correlative Data* was renamed *Comparison of Microbial and Physical Methods* to better reflect the purpose of the section. A new section pertaining to more complex container-closure systems was proposed, and various editorial changes were also incorporated. This chapter became official in USP 26 (34).

⟨1208⟩ Sterility Testing—Validation of Isolator Systems

The 1999 proposal for a new chapter on validation of isolator systems to be used in sterility testing (35) first appeared as an official guidance chapter in 2002 (36). This chapter was well received, but the AMB committee received occasional comments on potential improvements. A revision to this chapter was proposed late in 2004 (37) in response to these comments. The proposed revision of this general information chapter includes the replacement of references to sterilization with references to decontamination, thereby reflecting more accurately what is accomplished by treating the inside of an isolator with a process that eliminates viable bioburden. Other changes reflected the new ISO standards 14644-1, -2, -3, and -7.

⟨1209⟩ Sterilization—Chemical and Physicochemical Indicators and Integrators

This proposed new chapter, which previously appeared as an *In-Process Revision* in 1994 (38) and was subsequently cancelled, was substantially revised (39). The introductory section of this proposal was expanded to consider the use of chemical physicochemical indicators and integrators as alternate methods of testing for sterility of parametrically released products. In addition, the Class II medical device approval requirements are mentioned. The general sections on *Physicochemical Integrators*, on *Physicochemical Integrators in Moist Heat Sterilization* and on *Physicochemical Integrators in Ethylene Oxide Sterilization* were revised and expanded. The chapter became official in USP 27, First Supplement.

⟨1211⟩ Sterilization and Sterility Assurance of Compendial Articles

This chapter has lain neglected since it was created during the late 1980’s and is well in need of revision. The first serious revision to the chapter in almost 25 years appeared in the Sept–Oct 2004 issue of *Pharmacopeial Forum* (40). This proposed *In-Process Revision* provided numerous updates to the chapter. Lethality value (F_0) was introduced into the chapter. Updated taxonomic terms for biological indicator species were incorporated. Differences between biological indicator usages for moist-heat versus gas sterilization are discussed. In addition, the sections covering various modes of sterilization have been revised and updated as well as the inclusion of a new section on vapor phase hydrogen peroxide sterilization. Finally, discussion pertaining to Stage 2 of sterilization testing that was in the chapter was deleted to reflect the currently official chapter ⟨71⟩ Sterility Tests.

⟨1222⟩ Terminally Sterilized Pharmaceutical Products—Parametric Release

The USP proposed guidance chapter “⟨1222⟩ Terminally Sterilized Pharmaceutical Products—Parametric Release” was published as an *In-process Revision* in 2003 (41). This proposed new chapter, which previously appeared in *Pharmacopeial Previews* (42), was amended changes based upon comments received:

- All references to “sterility assurance level” (SAL) were restated using the positive exponent notation as part of a clarification of the SAL concept
- A section was added discussing three categories of general methods used for terminal sterilization (bioburden-based process, biological indicator/bioburden combined process, overkill process)
- The discussion pertaining to critical aspects of biological indicators used for sterilization validation was expanded and clarified
- The discussion regarding the *Sterilization Microbiology Control Program* was expanded and clarified
- The section on *Physicochemical Indicators and Integrators* was removed and incorporated into the proposed chapter (1209) *Sterilization-Chemical and Physicochemical Indicators and Integrators* (39)
- The discussion of physicochemical indicators under *Modes of Sterilization* (both for moist heat sterilization and ethylene oxide sterilization) has been removed and incorporated into the proposed chapter (1209).

This revision was included in the First Supplement to USP 2004 (43). However, several minor revisions were proposed in 2004 (44). These revisions included clarifying the use of the sterility assurance level (SAL) with aseptic processing and to revise the discussion of the number of spores required for the evaluation of a sterilization process. In addition, the exponent referring to SAL in the section *Radiation Sterilization* was to be changed from positive to negative. In addition, a number of editorial changes are also proposed.

⟨1223⟩ Validation of Alternative Microbiological Methods

The individual compendial assays in USP have allowed for alternative methods, as had the section *Procedures* under *Tests and Assays* in *General Notices and Requirements*, USP, but there has not been an informational chapter concerning how to perform the validation of these assays. The first publication of such a chapter appeared as a *Pharmacopeial Preview* in 2002 (45). This chapter was similar in many respects to the existent USP chapter “⟨1225⟩ Validation of

Compendial Methods.” However, as chapter (1225) is directed towards analytical chemistry assays, the AMB EC felt that it was necessary to develop a chapter specifically for validation of techniques other than the traditional methods (plate count, liquid growth, biochemical assay *etc*). This proposal borrowed heavily from portions of PDA Technical Report #33 on validation of rapid microbiological methods (46). The draft chapter defined three types of microbiological assay, qualitative, quantitative and identification. Each of these types had different requirements for validation.

This proposal was well-received, but there was significant comment on the need to clarify certain portions of the chapter. Therefore the AMB EC reworked the chapter and released it as an *In-process Revision* in 2003 (47). This proposed new chapter contained an expanded introductory section which was intended to clarify the special requirements of microbiological test validation relative to the data elements addressed in “⟨1225⟩ *Validation of Compendial Methods*.” In addition, several confusing tables were deleted, and the application of the terms “accuracy” and “precision” pertaining to qualitative microbiological tests were clarified. Finally, numerous editorial changes were made to simplify the text. These efforts met with mixed success and the chapter will continue to be revised before it becomes official. It is interesting to note that the *Pharm Eur* has recently released a draft informational chapter that differs from both the PDA Technical Report #33 and the draft efforts of USP (48).

⟨1231⟩ Water for Pharmaceutical Purposes

Although this chapter is not the responsibility of the AMB committee, it is included in this review of activities due to its inherent interest to the microbiologist. This chapter has been recognized as an authoritative resource for the pharmaceutical water treatment and analysis industry and is the only USP source of recommendations for the microbial of pharmaceutical waters applied in the regulatory arena. At the USP Open Conference on Microbiological Compendial Issues in 1996, attendees recommended that this chapter provide more information on water chemistry. Also, since the establishment of the chapter, it became evident that additional definitions, clarifications, water treatment methodologies, etc., would be useful. This

proposal was almost a complete rewrite of the existent chapter (49).

The proposed revision stimulated significant comment and after further consideration, the Pharmaceutical Waters Expert Committee made extensive changes to the proposed chapter. The revised version was published as an *In Process Revision* (50) and the expert committee will consider comments received.

⟨2021⟩ Microbial Enumeration Tests—Nutritional and Dietary Supplements

The proposed revisions to this chapter, which previously appeared in *Pharmacopeial Previews*, were forwarded to *In-Process Revision* in this publication (51). It was been substantially revised, with the following changes based upon comments received:

- The title was changed.
- Microbial identification methods were removed and placed into the new proposed general chapter ⟨2022⟩ Microbiological Procedures for Absence of Specified Microorganisms in Nutritional and Dietary Supplements (52), which also appeared in this issue of *PF*.
- The Preparatory Testing section was moved forward in the chapter and revised.
- Table 1 from the previous proposal was deleted.
- The Buffer and Media section was revised, with many media recipes removed and placed into the proposed chapter (2022).
- A section on Growth Promotion Testing was added.
- The sections on *Sampling* and *Test Procedures* were revised.
- The title of the table on most probable total count was revised.

The chapter became official in USP 27, First Supplement. Subsequent to that time, an erratum was published in *PF* 30(5) [Sept.–Oct. 2004] in which a table heading was corrected from “1, 0.1, 0.01” to “0.1, 0.01, 0.001”.

⟨2022⟩ Microbiological Procedures for Absence of Specified Microorganisms—Nutritional and Dietary Supplements

This topic was introduced in 1999 as a *Pharmacopeial Preview* (54), and was forwarded to *In-Process Revision* in 2003 (52) after substantial revision. The title was changed from “⟨2022⟩ Microbiological Procedures for Absence of Objectionable Microorganisms In Nutritional and Dietary Supplements.” to “⟨2022⟩ Microbiological Procedures for Absence of Specified Microorganisms—Nutritional and Dietary Articles.” This change reflects the experiences with harmonization of the Microbial Limits chapters and the realization that none of these chapters is, in fact, intended to provide methods for the demonstration of absence of all potential objectionable microorganisms (see discussion above). In addition to the title change, the use of Fluid Lactose Medium was eliminated, the *Preparatory Testing* section was revised and the *Test Procedures* section was revised and simplified. The chapter became official in USP 27, First Supplement.

⟨2023⟩ Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements

This proposed new chapter appeared as an *In-process Revision* in 2003 (54) which was the result of further consideration based on comments received about the previous version which appeared in *Pharmacopeial Previews* (55). The following changes based upon the comments received:

- The title was changed from “⟨2023⟩ Microbiological Attributes of Nonsterile Nutritional and Dietary Articles to ⟨2023⟩ Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements.”
- The introductory material was expanded, with further discussion of raw materials, pharmaceutical ingredients, and the active ingredients used in the manufacture of nutritional and dietary supplements.
- The discussion of microbiological monitoring was expanded as was the *Supplement Components* section which also contained further discussion pertaining to the *Enterobacteriaceae*.

- A table providing definitions for a range of botanical materials was added, and two tables containing recommended microbial limits were revised.

This version was incorporated into the *First Supplement* to USP 2004 (56). The Sept–Oct 2004 *PF* presented proposed revisions to the newly approved final version (57) which were proposed to clarify the discussion of the *Test for Aflatoxins* in the section *Absence of Objectionable Microorganisms*. It was also proposed to change the entry for Teas in Tables 1 and 2 to “Botanicals to be treated with boiling water before use.”

Stimuli to the Revision Process

The *Pharmaceutical Forum* provides a mechanism for interested parties in the field to publish scientific articles of interest to the audience. During the 2000—2005 revision cycle there were several articles on topics handled by the AMB.

Antimicrobial Efficacy, Microbial Limits and Sterility Testing

The Effects of Antimicrobial Preservatives On Organisms Derived from Fresh Versus Frozen Cultures (58).

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 significant 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.

An Alternative Methodology for the General Test Chapter Microbial Limit Tests (61) (59).

The authors present a simple methodology which they claim “provides better sensitivity while reducing the overall cost of the test. Enrichment for all objectionable organisms is carried out in a rich, nonselective medium (trypticase soy broth). If growth is present in the enrichment medium following a 48-hour

incubation period, the sample is streaked on MacConkey Agar Medium and Mannitol-Salt Agar Medium. All isolates from the media plates are then identified to the species level. Results demonstrate that this method is equivalent, and in one case superior, to the current USP method.”

Comparison of Microbiological Testing Practices in Clinical, Food, Water and Pharmaceutical Microbiology in Relation to the Microbiological Attributes of Nutritional and Dietary Supplements (60).

The author compares the microbiological testing practices employed in clinical, food, water and pharmaceutical microbiology. This evaluation was done as part of the preparatory work to the development of the “Microbial Limits of Nutritional Supplements” chapters under development at the time. This article serves as a useful benchmarking review of the test method selection, validation practices, quality control, sampling plans, and specification setting techniques in the various disciplines.

Review of the Media Section and Incubation Conditions for the Compendial Sterility and Microbial Limit Tests (61).

The author reviews the media and incubation conditions recommended for the USP (71) Sterility Tests and (61) Microbial Limits Tests. This review presents an overview of the history of the tests, and explores their limitations. The author ends by providing specific recommendations for the compendial application of the tests.

Rapid Microbiological Methods and Process Analytical Technology

The Role of Rapid Microbiological Methods Within the Process Analytical Technology Initiative (63).

The authors discuss the opportunities presented by rapid microbiological methods for in-process and finished-product testing. Special attention is paid to the current FDA Process Analytical Technology Initiative and how this provides an opportunity for regulatory acceptance of the newer technology. The

authors describe some of the rapid microbiology technologies and suggest where they might be applied in support of manufacturing.

Microbial Identification in the Pharmaceutical Industry (63).

The authors review microbial identification methods that are available to support compendial testing. Microbial identification methods included screening techniques, biochemical identification, and the different technologies available for use today. These technologies include phenotype characterizations (biochemistry, fatty acid analysis, serological techniques, etc) as well as genotypic methods (ribotyping and DNA sequencing). The authors recommend removing the discussion of microbial identification from the proposed guidance chapter “(1227) *Validation of Alternative Microbiological Methods*.”

The FDA Process Analytical Technology (PAT) Initiative—An Alternative Pharmaceutical Manufacturing Practice (aPMP) (64).

This article was prepared by USP’s Project Team 18 (PAT) and presents an overview of FDA’s Process Analytical Technology (PAT) initiative from the perspectives of industry and the U.S. Pharmacopeia. The project team members . . . “outline the scope of PAT as a scientific, risk mitigation based approach for process-centered quality control techniques within an alternative Pharmaceutical Manufacturing Practice (aPMP) environment. The authors discuss the various elements of the PAT initiative, explore USP’s initial role in PAT, and highlight the key PAT concepts. Finally, this article presents a proposed list of terms that have been identified as members of a glossary for PAT analysis.”

Other Topics of Interest

Sterilizing Filtrations with Microporous Membranes (65).

The authors review the nature and uses of membrane filtration in liquid sterilization practices. Starting with the historical stance that the nominal pore size rating told the user

everything necessary about the sterilizing capabilities of the membrane, the authors explore a variety of other contributing factors. Their conclusions were that the actual capabilities of the membrane were influenced by “. . . the physicochemistry of the fluid; pH, viscosity, and chemical composition, and its compatibility with the filtration matrix. The filtration conditions are also influential: the filtration velocity/flow rate, the differential pressure across the filter membrane, and the duration of the filtration. Additionally, the surface properties of the filtration matrix, particularly its propensity for adsorptions, and the size and surface properties of the particles being filtered are important.”

The USP Perspective to Minimize the Potential Risk of TSE Infectivity in Bovine-Derived Articles Used in the Manufacture of Medical Products (66).

This article examines on the potential risk of acquiring transmissible spongiform encephalopathy through the use of bovine-derived articles in the manufacture of medical products, and on risk-reduction strategies to minimize this risk. The authors look at two main topics: a review of the illness and risk-reduction strategies. The discussion of risk-reduction strategies is basically an overview of current guidelines that may ultimately be included in a new general information chapter on the subject.

Significant Digits and Rounding. (67).

This article was prepared in response to a need identified by the USP Biostatistics Expert Committee (BST). This presentation might be included in a new general information chapter. The author presents an introduction to data formats, and to documentation of those data. General guidance is then provided to rounding issues, and to significant digits, and how they are to be applied to compendial specifications. This article is particularly interesting given the current work of this committee on the proposed new guidance chapter “(1010) *Analytical Data-Interpretation and Treatment*” (68, 69).

Conclusions

This has been a very busy revision cycle for the AMB Expert Committee. The addition of several new informational chapters, the on-going harmonization discussions, and the need to revise several older informational chapters provides many opportunities for the USP AMB Expert Committee. It is also evident that the USP is engaged in an iterative process of continuous improvement, with all chapters open to review. In keeping with the USP policy of ongoing, continuous revision, several new chapters are under development along with revision of established chapters. This process is greatly facilitated by the comments received by USP from workers in the field and helps to keep the USP current. The need for change will not end with the 2000–2005 cycle: with the development of newer microbiological technologies, the ongoing process of harmonization and initiatives from regulatory agencies, the 2005–2010 cycle promises to be an active one.

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