

# Activities of the USP Microbiology and Sterility Assurance Expert Committee During the 2005–2010 Revision Cycle

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## Abstract

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This article is a comprehensive review of the published activities of the Microbiology and Sterility Assurance Expert Committee (MSA EC) of the United States Pharmacopeial Convention (USP) Council of Experts for the 2005–2010 revision cycle. This cycle involved the completion of several harmonization efforts, including Sterility Tests, Microbial Limits Tests, and the Bacterial Endotoxins Test. In addition, USP continued in its leadership of laboratory practices with informational chapters (both new and revised) in several areas of laboratory activity.

The EC had 9 new general chapters under development and managed 7 monographs, 3 of which were new this cycle. In addition to reviewing the changes accomplished, this article discusses the rationale for many of the changes and some background information about new initiatives now underway. Where appropriate, the authors discuss changes in USP that did not fall under the direct purview of the MSA EC (whose responsibility included 7 monographs and 37 general chapters) but are of interest to the microbiology community.

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## Introduction

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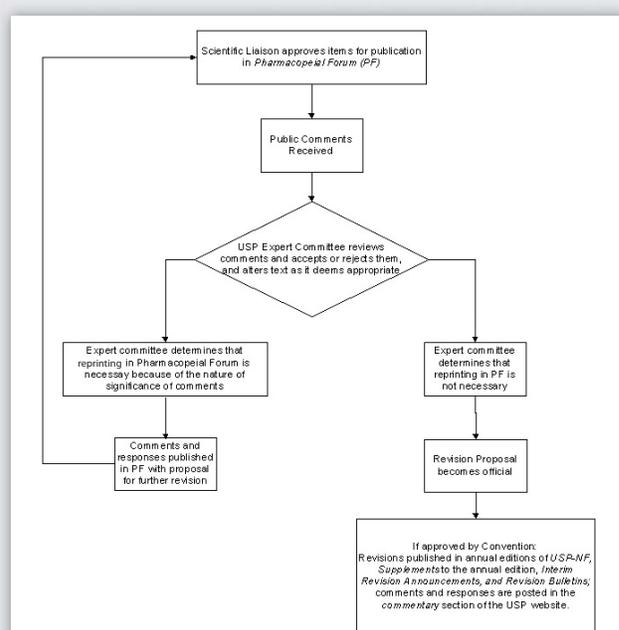
The 2005–2010 revision cycle of the USP Microbiology and Sterility Assurance Expert Committee (MSA EC, the EC) included the completion of several harmonization efforts as well as the beginning of new efforts. In addition to creating new chapters, the EC actively updated older chapters and worked toward international harmonization of referee chapters and critical monographs (see USP 2007aa for a discussion of the harmonization process). Several of the publications that occurred in the 2005–2010 revision were actually work initiated by the expert committee during the 2000–2005 revision cycle (Sutton 2001), and these are included in this review with the acknowledgement of the hard work of the previous committee, many of whose members continued to serve during the 2005–2010 cycle. The MSA EC of the USP Council of Experts is responsible for general information chapters dealing with microbial assays and microbial control of processes and environments. The MSA EC develops or revises chapters as deemed necessary for the advancement of pharmaceutical microbiology. Their responsibility does not extend to drug or product monographs, which are handled

by other ECs, although the MSA EC does support the development of microbial requirements for all monographs via consultation with the relevant monograph committees. Similarly, antibiotics are handled by a separate expert committee, as are the monographs and informational chapters on pharmaceutical waters.

USP includes two distinct types of chapters: standards and informational 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. Second, they are not intended to be batch release methods. They are standards that describe minimal expectations for the quality of medicinal products. If, for example, a pharmaceutical product does not fulfill the requirements of the appropriate USP monograph, then it is “misabeled” or “adulterated,” based on the provisions of the Federal Food, Drug, and Cosmetic Act, and the Food and Drug Administration (FDA) can take enforcement actions. Because of this legal status of compendial tests, changes to any chapter or monograph are made only after careful consideration, including public review and comment. Similarly, the general information chapters in USP are widely held as a reference guide to industry, and changes to the official chapter are similarly introduced very carefully, again after a period of public review and comment.

The revision process of USP is designed to provide the maximum opportunity for input into the deliberative process. This process undergoes constant review, and the 2005–2010 revision cycle saw some significant changes in terms of time limits and procedures for implementing different aspects of the process [see USP Project Team on Compendia/Process Improvements (PT 19) 2005]. The process as it is currently implemented is presented in Figure 1 (based on the similar figure in the USP Mission and Preface). Interested parties, including members of the EC, propose a new chapter or changes to current chapters. These proposals then are forwarded to the EC for review and consideration. Following review, the proposals appear on-line in Pharmacopeial Forum (PF) (USP 2009j). Previously, if the proposal was a new chapter or if significant changes were being made to an existing chapter the proposal would appear as a Pharmacopeial Preview, but this stage of the process has been removed (USP 2009h). Under the current procedures, the proposal is published in the In-Process Revision section of PF or as a Stimuli to the Revision Process article (commonly termed a Stimuli article).

At this stage public (industry, academia, and government) participation becomes critical. As Figure 1 clearly shows, if there is no input into the process, the text that represents the EC’s best effort will become official. This generally results in a less than optimal result (for a discussion of a recent example of this situation, see Sutton 2009a). The process is designed to incorporate public comments, and they are an essential part of the process. After public comments are received, the EC reviews the comments and incorporates them, if warranted, into a revised chapter proposal. If the changes are significant, this proposal will appear again in PF for comment. Subsequent public comments and revisions to the chapter will continue to appear in PF until the EC is satisfied that the proposal is ready to become official. At that point, the EC ballots on and approves the chapter. If approved, the chapter appears in one of



**Figure 1. The Public Review Process of USP (from USP–NF, Mission and Preface. Used with permission from USP; all rights reserved).**

the semi-annual Supplements or the annual publication of the book. Supplements are the mechanism used to update USP–NF between the annual publications of the book.

If a significant number of comments are received after publication of the approved 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 that responds to changing regulatory and stakeholder needs. A summary of the activity is provided in Table 1. 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, PF 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 2005–2010 revision cycle, PF included several articles of particular interest to the work of the Microbiology Subcommittee. Stimuli articles are designed to promote discussion of new ideas or provide data to assist the EC in improving chapters in USP and have been used in the revision process by the MSA EC. A list of the relevant articles is provided in Table 2, and each is discussed later in this article.

Clearly, USP is continuously evolving, and stakeholders can contribute to further changes in the structure, content, and focus of USP–NF. For example, an excellent review of the chapter structure of USP and how it fits into the global regulatory environment was recently presented (USP General Chapter Management Team 2009), as was a description of the revision of USP monographs to

**Table 1. Summary of Activity**

	In-Process Revision	Finalized	Total
<55> Biological Indicators—Resistance Performance Tests: Total Viable Spore Count	1	0	1
<61> Microbial Examination of Nonsterile Products: Microbial Enumeration Tests	2	2	4
<62> Microbial Examination of Nonsterile Products: Tests for Specified Microorganisms	2	2	4
<63> Mycoplasma Tests	1	0	1
<71> Sterility Tests	1	1	2
<85> Bacterial Endotoxins Tests	1	1	2
<610> Alternative Microbiological Sampling Methods for Nonsterile Inhaled and Nasal Products	1	0	1
<1072> Disinfectants and Antiseptics	1	1	2
<1111> Microbiological Attributes of Nonsterile Pharmacopeial Articles	1	1	2
<1116> Microbial Control and Monitoring Environments Used for the Manufacture of Healthcare Products	2	0	2
<1117> Microbiological Best Laboratory Practices	1	2	3
<1113> Microbial Characterization, Identification and Strain Typing	2	0	2
<1208> Sterility Testing—Validation of Isolator Systems	0	1	1
<1211> Sterilization and Sterility Assurance of Compendial Articles	1	1	2
<1222> Terminally Sterilized Pharmaceutical Products—Parametric Release	0	1	1
<1223> Validation of Alternative Microbiological Methods	1	1	2
<1227> Validation of Microbial Recovery from Pharmacopeial Articles	0	1	1
Biological Indicator Monographs	0	2	2

“performance-based” monographs that reduce the reliance of the industry on USP test methods and encourage development of specific methods for the product under test (USP Small Molecules Collaborative Group 2009).

This review examines the published activity by chapter in numerical order with the exception of the Microbial Limits chapters that will be considered together. This number sequence is important in its own right because 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. Chapters numbered from >1000 are general information chapters and are not intended to be enforced. Chapters beginning at <2000> are general information chapters devoted to nutritional supplements. Chapters reviewed in this article include:

- <55> Biological Indicators—Resistance Performance Tests: Total Viable Spore Count
- <61> Microbial Examination of Nonsterile Products: Microbial Enumeration Tests
- <62> Microbial Examination of Nonsterile Products: Tests for Specified Microorganisms
- <63> Mycoplasma Tests

**Table 2. Stimuli Articles Published**

Stimuli Article	Reference
A Rapid Capillary Electrophoresis Method for the Detection of Microbial Contamination: An Alternative Approach for Sterility Testing. Bao Y, et al	36 (6):1798–1805. 2010
General Chapter Management in the 2010–2015 Cycle. USP General Chapter Management Team	35 (5):1372–1379. 2009
Liquid-filled Gelatin Capsules. Marques MRC, et al	35 (4):1029–1041. 2009
Acceptable, Equivalent, or Better: Approaches for Alternatives to Official Compendial Procedures. Hauck W, DeStefano A, et al	35 (3):772 – 778. 2009
Redesign of the United States Pharmacopeia and National Formulary. Cecil T.	34 (6):1638 – 1640. 2008
Making Sense of Trueness, Precision, Accuracy, and Uncertainty. Hauck W, Koch W, et al	34 (3):838 – 842. 2008
The Role of Container–Closure Systems in Stability Testing for Climate Zone IV. Lockhart H, Selke S, et al	32 (2):688 – 694. 2006
Comments on “Compendial Calculations: Improving the Calculations in USP–NF”. USP	31 (6):1756 – 1757. 2005
Compendial Calculations: Improving Calculations in USP–NF. Travis P, Heck K, et al	31 (5):1749 – 1755. 2005
The Development of Chapter <1235> Vaccines and Vaccine Test Methods. Garfinkle BD, et al	31 (5):1533–1538. 2005
Microbial Testing for Orally Inhaled and Nasal Drug Products. USP Project Team 7	31 (4):1258–1261. 2005
RSD and Other Variability Measures of the Lognormal Distribution. Tan CY	31 (2):653–655. 2005
The USP Revision Process: Recommendations for Enhancements. Bishara RH, et al	31 (2):656–661. 2005

- <71> Sterility Tests
- <85> Bacterial Endotoxins Test
- <610> Alternative Microbiological Sampling Methods for Nonsterile Inhaled and Nasal Products
- <1072> Disinfectants and Antiseptics
- <1111> Microbial Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use
- <1112> Application of Water Activity Determination to Nonsterile Pharmaceutical Products
- <1113> Microbial Characterization, Identification, and Strain Typing
- <1116> Microbial Control and Monitoring Environments Used for the Manufacture of Healthcare Products
- <1117> Microbiological Best Laboratory Practices
- <1208> Sterility Testing—Validation of Isolator Systems
- <1211> Sterilization and Sterility Assurance of Compendial Articles
- <1222> Terminally Sterilized Pharmaceutical Products—Parametric Release
- <1223> Validation of Alternative Microbiological Methods
- <1227> Validation of Microbial Recovery from Pharmacopeial Articles

Following is a brief review of each chapter:

## <55> Biological Indicators— Resistance Performance Tests: Total Viable Spore Count

The January–February 2004 issue of PF (USP 2004f) included a proposal to revise the sections on Total Viable Spore Count and D-Value Determination to add requirements specified in the proposed new monographs for Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Metal or Plastic Carriers (USP), and Biological Indicators for Moist Heat, Dry Heat, and Gaseous Modes of Sterilization, Liquid Spore Suspensions. These tests are designed to be used by the manufacturers of the biological indicator, not as an acceptance test by the user of the commercially prepared biological indicator. A useful review of this subject is available (Nyberg 2006).

### *The Harmonized Microbial Limits Tests*

An important accomplishment during the 2005–2010 revision cycle was the completion of the chapter harmonization efforts that began during the 1995–2000 cycle. Approximately 60% (12 of 21) of published revisions and final draft announcements were traceable to this effort. An important point of contention for the MSA EC was the Microbial Limits Tests, which suffered from technical issues related to media choices and availability (Sutton 2006). One of the issues encountered with this harmonization effort was the apparent lack of industry interest in the process, at least until the chapters were near finalization (Sutton 2009a). Late public responses led to a postponement of the chapters from 2007 to 2009 (USP 2007d).

The major difficulty in ignoring proposed changes to USP is that these changes potentially have important effects on the work load in the lab (Sutton 2009b), to say nothing of the role this test plays in raw material acceptance and finished product release under current good manufacturing practices (cGMP) (Bomblies 2007, Cundell 2005, Karnam 2008, Madden 2005). One of the take-home messages from this experience is that USP depends on input from the field for chapter revisions, and if industry fails to participate no one is happy with the result.

The harmonized microbial limits tests are actually three separate chapters that deal with enumeration, absence of specified organisms, and general information about the microbial quality of nonsterile products. The changes to these chapters are outlined next:



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“ The revision process of USP is designed to provide the maximum opportunity for input into the deliberative process. ”

## <61> Microbial Examination of Nonsterile Products: Microbial Enumeration Tests

The first publication during the 2005–2010 revision cycle was a finalized chapter in 2006 (USP 2006a). This delay afforded the opportunity to make some minor changes (USP 2007e, USP 2008a), and Controls were enhanced with an expansion of the role of the negative control in the test. Compared to previous versions, this revised chapter includes an expanded discussion of the Most Probable Number method of enumeration (Sutton 2010a). The harmonized chapter became official in 2009 (USP 2009a).

## <62> Microbial Examination of Nonsterile Products: Tests for Specified Microorganisms

The publication schedule of USP <62> was identical to that of <61>. The 2006 publication (USP 2006b) was followed by annual, final (i.e., published and official) versions in 2007 and 2008 (USP 2007f, USP 2008b). Changes to the text paralleled those involving the negative control in chapter <61>. In addition, in the 2008 final version minor changes were made to the quality control of the media (particularly the removal of *E. coli* as a growth promoting and indicative check on XLD Agar). The harmonized chapter became official in 2009 (USP 2009b).

## <1111> Microbiological Attributes of Nonsterile Pharmacopeial Articles

The third chapter in the Microbial Limits Tests is a general information chapter that has appeared twice in final form (USP 2006c, USP 2007g). It provides an indication that the enumeration test is subject to variability (which should come as a surprise to no one in the lab), and so a twofold excess in recovery is acceptable. This information also is included in chapter <61>. Analysts should note the admonition to check the “the significance of other microorganisms recovered” and the provision of a rudimentary risk-analysis scheme, which is particularly important in the context of US cGMP requirements for “objectionable organisms”:

- Each lot of a component, drug product container, or closure that is liable to microbiological contamination that is objectionable in view of its intended use shall be subjected to microbiological tests before use [21 CFR 211.84(d)(6)].
- Appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, shall be established and followed [21 CFR 211.113(a)].
- There shall be appropriate laboratory testing, as necessary, of each batch of drug product required to be free of objectionable microorganisms [21 CFR 211.165(b)].

This correlation of USP and cGMP is not accidental and completes a process that began in the 1980s when contaminated inhalants led to concerns about the microbial limits test (Kuhn 1982, USP 1982). Clearly, the compendia and FDA are in alignment regarding the difference between the compendial monograph requirements for “absence of specified organisms” and the cGMP requirement for “absence of objectionable organisms” in nonsterile finished dosage forms.

## <63> Mycoplasma Tests

This new chapter proposal contained methods to detect Mycoplasma contamination of test articles, tissues and/or cell cultures used to produce test articles, or any other material in which Mycoplasma contamination was suspected (USP 2009c). This chapter is an early effort focusing on culture methods for mycoplasmal detection.

A validated procedure based on nucleic acid amplification techniques, an enzymatic activity-based method, or other similar tests, can be used to detect mycoplasma provided the assay can be shown to be comparable to both a) agar and broth media procedure and b) the indicator cell culture procedure. The proposed chapter received a modest number of comments, most of which were incorporated into an improved version that became official in USP 33–NF 28 Reissue. To facilitate possible future harmonization, the chapter is largely consistent with the corresponding chapters in the European and Japanese pharmacopeias. Where it differs from the other pharmacopeias, it is consistent with FDA/CBER guidelines for Mycoplasma testing.

## <71> Sterility Tests

The harmonization of the Sterility Tests among the three regional pharmacopeias was handicapped by the nearly canonical status this test enjoys in the industry. After the test was harmonized by the Pharmacopeial Discussion Group, the final version was published early in the past revision cycle and then revised several times during the past revision cycle (reviewed in the previous article of this ongoing series—see Sutton 2005). As so often happens, the “final harmonized” version of the Sterility Test was amended and was published as final twice in the 2005–2010 revision cycle. A revision that appeared in 2008 (USP 2008b) removed several inconsistencies and retitled the section on demonstrated microbial recovery to Method Suitability. The issue was that compendial tests are by definition valid, and therefore validating a validated test was a bit confusing. For the record, this section over time has been called Bacteriostasis/Fungistasis, Validation, and now Method Suitability.

After this effort, the chapter was considered completely harmonized except for regional differences allowed under the harmonization procedure. However, the International Conference on Harmonization (ICH) Q4B working group noted that some words remained different among the regional versions and insisted on complete harmonization. This harmonized version appeared in 2009 (USP 2009d). The European Pharmacopoeia (Pharm Eur) has published a nonmandatory chapter on guidelines for the use of the harmonized sterility test to address their concerns (Pharm Eur 2009). One hopes this chapter, which appears in USP as <71>, is, in fact, harmonized to everyone’s satisfaction.

The harmonized compendial sterility test continues to generate interest as well.

Cardoso et al. examined several variants of benign solvents to assist in testing ophthalmic ointments and found that isopropyl palmitate far less toxic than the commonly used isopropyl myristate (Cardoso 2006). A review of the test appeared in 2006 (Mentel 2006) when researchers examined the test from an operational perspective. Two articles examined limitations of the test: In the first, a Spanish group showed significant contamination of a vaccine product by *Clostridium sordellii* (Télez 2006) that was not identified by the harmonized test but became apparent after 60 days of incubation (as opposed to the 14 days

of the test). It should be noted that the limitations of the test are well known and have been documented for decades (Bryce, 1956). The difficulty lies in improving it (Moldenhauer and Sutton, 2004).

## <85> Bacterial Endotoxins Tests

Unlike the Sterility tests, the finalized version of this test that became effective in 2002 (USP 25–NF 20) did not have any residual

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nonharmonized elements. To improve clarity for users it was further revised in 2007 (USP 2007h), and the final version was published in 2009 (USP 2009e). At present, additional minor changes are being made to meet the objective of regulatory interchangeability.

Future revision of the harmonized Bacterial Endotoxin Test chapter seems likely. From a clinical perspective, Szathmary calls into question accepted endotoxin limits for parenteral products, noting that many patients in a compromised state are exposed to several different sources of endotoxin introduction via the parenteral route (Szathmary 2005). Because of this likely duplication of sources, he presents a thought-provoking analysis suggesting that commonly accepted endotoxin limits may in fact be too high.

A more important reason for revision of the harmonized test may be advances in technology. One product offering is based on a recombinant Factor C (Thorne 2010), and another is a highly automated endotoxin test kit (Jimenez 2010). Both of these new technologies are under evaluation by the industry.

In addition to the promise of increases in efficiencies and decrease in costs by the use of the Bacterial Endotoxin Test and perhaps some of the new technologies is the current regulatory interest in Quality by Design (QbD). Williams argues persuasively that the use of the BET within the process improvement framework can yield significant advantages (Williams 2009).

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## <610> Alternative Microbiological Sampling Methods for Nonsterile Inhaled and Nasal Products

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This proposed new general test chapter provides special approaches for sampling of either low- or high-content inhaled or nasal dosage forms (USP 2010a). Its goal is to encourage methodologies that employ aseptic techniques and are conducted under environmental and other conditions that are appropriate for aseptic sampling. It also provides some guidance about sample sizes. This new chapter is currently available for public comments. Further information about the general topic of microbial testing of inhalants is available in a Stimuli article (USP Project Team 7 2005).

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## <1072> Disinfectants and Antiseptics

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The official chapter first appeared in Supplement 2 of USP 29 (USP 2006d). This chapter provided a set of definitions and then presented a classification scheme for the agents based on their chemical nature, along with proposed uses (germicide, antiviral, sporicide, etc). Following sections included guidance on selection of an antiseptic for hand and surgical site selection, 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. The chapter also provided guidance about disinfectant

challenge testing and the role of disinfectants in a cleaning and sanitization program.

Once official, this chapter generated comments from the industry, and USP decided to revise portions of the chapter for clarity (USP 2009f). After USP received no further significant comments, the revised text was finalized in 2010 (USP 2010b).

One of the significant changes in the 2009 revision was a strengthening of the discussion of disinfectant rotation in order to clarify the need to have a qualified disinfectant for the facility and its microflora and to rotate this agent with a qualified sporicide. The science behind this recommendation, and the need to qualify the disinfectants for the specific facility, can be found in two independent review articles (Sutton 2005b, Wallis 2007).

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

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This new informational chapter first appeared in 2002 (US 2002) with the goal of providing information about the influence of water activity on product formulation susceptibility to microbial contamination. The chapter discusses the potential for improving product preservation by maintaining low water activity. The chapter included one table that provided data about water activity requirements for the growth of a range of microorganisms, and another table gave strategies for microbiological testing based on product water activities. In addition, it presented a method for the measurement of water activity. This chapter generated a notable amount of discussion, and a revision was published in 2004 (USP 2004a). Changes on the basis of comments received included a revision of the chapter title and clarification of some aspects of risk analysis related to water activity. In particular, changes emphasized the importance of container-closure integrity in maintaining the water activity level during product shelf life. Other changes explicitly acknowledged the ability of more resistant microorganisms to persist in drug products with low water activity. Finally, the chapter stated clearly that reduced microbial limits testing must be justified by risk assessment and not solely by water activity determinations. This text became official in Supplement 2 of USP 29 (USP 2006e)

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## <1113> Microbial Characterization, Identification, and Strain Typing

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This is a new informational chapter in USP. Initially intended to be a discussion of the qualification of microbial identification technologies, the topic was part of the development of informational chapter Validation of Alternative Microbiological Methods <1223>. However, this topic was removed during the 2000–2005 cycle for separate treatment (Sutton 2004).

The proposed draft version published in 2009 was titled simply Microbial Identification and covered purification techniques,

preliminary screening techniques, phenotypic and genotypic identification techniques, polyphasic techniques, and some background in verification and qualification of the technologies (USP 2009g). This proposal elicited strong response from the field. A revised proposal was published (USP 2010c) and addressed these concerns. This revision shifted the emphasis of the chapter, which is reflected in its new title, Microbial Characterization, Identification, and Strain Typing.

This informational chapter is significant to the QC microbiology lab because it offers guidance about the qualification of the relevant equipment and assists in the determination of appropriate technology. Accurate and dependable bacterial identification is important to the success of the QC microbiology lab (Cundell 2006). The accurate identification of microbial contaminants is important not only from an internal quality perspective, but it often is central to the successful completion of product investigations and is frequently a consideration during product recalls. This challenge has only increased with the proliferation of nucleic-acid-based technologies and the changes in microbial taxonomy that have resulted (Pace 2009). An important example is the re-assignment of the ATCC standard strain 16404 from *Aspergillus niger* to *Aspergillus brasiliensis* (Houseknecht 2010), which then required updates of many USP chapters that cited ATCC 16404 as a control strain.

## <1116> Microbial Control and Monitoring Environments Used for the Manufacture of Healthcare Products

As described in the previous article in this series (Sutton 2005a) the revision of chapter <1116> has been a contentious one for the USP Microbiology committee. A new text was proposed in 2005 and incorporated some significant deviations from the extant text (USP 2005a). For starters, the proposed title was changed to include the clear separation of the concepts of control and monitoring in clean room environments. In addition, the new text included significant changes in the regulatory background of aseptic environmental monitoring, and this proposal eliminated reference to the withdrawn Federal Standard 209 E. In addition, many of the sections and tables of this information chapter have been revised:

- Table 5 and sections on Operational Evaluation of the Microbiological Status of Aseptically Filled Products in Clean Rooms and Other Controlled Environments and an Overview have been eliminated
- New sections were added:

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**Table 3. Articles Published on Sterility Assurance by USP Committee Members**

Agalloco, J, J Akers and R Madsen. 2004. Aseptic Processing: A Review of Current Industry Practice. <i>Pharm Technol.</i> Oct:126–150.
Agalloco, J et al. 2004. Some Further Comments on Aseptic Processing Guidance, or the Fool on the Hill. <i>PDA J Pharm Sci Technol.</i> 57(5):324–330.
Agalloco, J and J Akers. 2005. Aseptic Processing: A Vision of the Future. <i>Pharm Technol.</i> (Asept Proc):s16–23.
Agalloco, J. 2005. Importance of Background Microbial Levels in the Manufacture and Testing of Sterile Products. <i>Pharm Technol.</i> April:74–80.
Agalloco, J. 2005. Managing Aseptic Interventions. <i>Pharm Technol.</i> March:56–66
Agalloco, J., Akers, J., et al. 2007. Choosing Technologies for Aseptic Filling: "Back to the Future, Forward to the Past?." <i>Pharm Eng.</i> 27(1):8 – 16.
Agalloco, J and J Akers. 2007. The Truth About Interventions in Aseptic Processing. <i>Pharm Technol.</i> (Asept Proc):s8–s11.
Agalloco, J., Akers, J., et al. 2009. Risk Management, cGMP, and the Evolution of Aseptic Processing Technology. <i>PDA J Pharm Sci Technol.</i> 63(1):8 – 10.
Agalloco, J and Akers, J. 2010. The "Myth" Called Sterility. <i>Pharm Technol.</i> Bioprocess Sterile Manufact Issues:s44–s45.
Agalloco, J and J Akers. 2011 Revisiting Interventions in Aseptic Processing. <i>Pharm Technol</i> 35(4):69–72.
Agalloco, J and TH Meltzer. 2004. Relative Filter Pore and Microorganism Sizes: The Conflict Between Science and Marketing – Pictorial Review. <i>Am Pharm Rev.</i> 7(5):54–61.
Agalloco, J and TH Meltzer. 2004. Relative Filter Pore and Microorganism Sizes: The Conflict Between Science and Marketing – Pictorial Review. <i>Am Pharm Rev.</i> 7(5):54–61.
Agalloco, J. 2007. Understanding Overkill Sterilization: An End to Confusion. <i>Pharm Technol.</i> (Asept Proc):s18–s25.
Agalloco, J., Akers, J., et al. 2009. Revisiting the Moist Heat Sterilization Myths. <i>PDA J Pharm Sci Technol.</i> 63(2):89 – 102.
Akers, J and J Agalloco. 2005. Risk Analysis for Aseptic Processing: The Akers – Agalloco Method. <i>Pharm Technol.</i> 29(11):74–88.
Akers, J. 2007. Charting Process Improvement in Sterile Product Manufacturing. <i>Pharm Technol.</i> 31(7):75 – 81.
Akers, J and J Agalloco. 2006. The Simplified Akers–Agalloco Method for Aseptic Processing Risk Analysis. <i>Pharm Technol.</i> 30(7):60–76.
Akers, J. 2010. Risk and Scientific Considerations in the Environmental Monitoring of Isolators in Aseptic Processing. <i>Am Pharm Rev.</i> 13:92–96.
Akers, J. 2011. Environmental Monitoring in Isolators – Time for a New Path Forward. <i>Am Pharm Rev</i> 14(2):18–25.
Cundell, AM. 2004. Microbial Testing In Support of Aseptic Processing. <i>Pharm Technol.</i> 6:58–66.
Izumi, Y. and Akers, J. 2009. The Impact of Automation on Aseptic Processing. <i>Pharm Technol.</i> pp s34 – s37.
Langille, S and D Hussong. 2004. Status and Direction of Aseptic Processing in the United States – 2004. <i>Am Pharm Rev.</i> 7(6):28–54.
Sutton, S. 2009. USP<1211>: The Compendial Informational Chapter on Sterility Assurance Origins and Future Direction. <i>Pharm Technol.</i> Ster Manufact:s16 – s21.

5 Physical Evaluation of Contamination Control Effectiveness—this section discussed ISO14644-1 and the need for the proper functioning of the clean room as a facility for contamination control

5 Sample Volumes for Active Air Monitoring—discussed the relationship among time of sample, volume of air sampled, dessication of the media, and sensitivity of the assay

Although it was not widely commented upon at the time, this proposed revision introduced a new measure, incidence frequency. An important problem with analysis of environmental monitoring data is that the data are well below the recognized Limit of Quantification for plate counts (25–30 CFU/plate) and so are wildly variable and yield

**Table 4. Articles on Alternate (Rapid) Microbiological Methods by Committee Members**

Sutton, SVW. 2005. Validation of Alternative Microbiology Methods for Product Testing: Quantitative and Qualitative Assays. <i>Pharm Technol.</i> April :118–122.
Hussong, D and R Mello. 2006. Alternative Microbiology Methods and Pharmaceutical Quality Control. <i>Am Pharm Rev.</i> 9(1):62–68.
Farrington, JK. 2006. Microbiological Method Validation (or is it Suitability?) Is this an Attempt to Prove the Impossible? <i>Am Pharm Rev.</i> 9(3):16–20.
Cundell, AM. 2006. Opportunities for Rapid Microbial Methods. <i>Am Pharm Rev.</i> 9(7):50–56.
Cundell, AM. 2006. Opportunities for Rapid Microbial Methods. <i>Eur Pharm Rev.</i> 1:64–70.
Cundell, T. 2006. Top Five Challenges Facing Pharmaceutical Microbiologists. <i>Am Pharm Rev.</i> 9(4):30–34.
Porter, D. 2007. Qualification, Validation, and Verification. <i>Pharm Technol.</i> 31(4):146 – 154.
Porter, DA. 2007. Review of USP Chapter <1223> Validation of Alternative Microbiological Methods Presented at the 2007 Global Conference on Pharmaceutical Microbiology. <i>Am Pharm Rev.</i> 10(3):76–81.
Porter, D. 2007. Review of USP Microbiology Chapters. <i>PDA Letter.</i> 18(2):1, 13–16.

imprecise estimates (Sutton 2007). This leads to notable difficulties in interpreting environmental monitoring data (Hussong and Madsen 2004, Farrington 2005, Sutton 2010b). One method of dealing with uncertain quantitative data is to treat it as qualitative data. In this approach, analysts recognize that 2 CFU is not materially different from 7 CFU on a plate, but the fact that there are colonies on the plate at all is the key fact. In effect, analysts are counting non-zero events. The 2005 draft chapter provides some basic information about how to address these issues. Readers are referred to Caputo's statistical treatment of this problem (Caputo 2004). Based on comments received from the industry, the EC published a second draft revision to this chapter in 2010 (USP 2010d). This was broadly similar to the first proposed revision and included increased emphasis on the renamed frequency of detection (as opposed to incidence frequency) of microbial contaminants in the aseptic core. The expected levels of detection frequency were unchanged from the 2005 draft because no comments were received regarding their appropriateness.

Environmental monitoring continues to be especially contentious not only in industry but also within the committee. A brief review of recent committee member publications shows the range of opinions (Akers 2006, Sutton 2010e, Tidswell 2006, Tidswell 2010). This area promises to be one of continuing development.

## <1117> Microbiological Best Laboratory Practices

This new chapter was published in its final form in Supplement 2 of USP 29 (USP 2006f) and contained information about the following topics:

- Media preparation and QC
- Maintenance of microbiological cultures
- Maintenance of laboratory equipment
- Laboratory layout and operations
- Training of personnel
- Documentation

- Maintenance of laboratory records
- Interpretation of laboratory results

The purpose of this chapter was that good laboratory practices in a microbiology laboratory should consist of activities that depend on several principles: aseptic technique, control of media, control of test strains, operation and control of equipment, diligent recording and evaluation of data, and training of the laboratory staff. Because of the inherent risk of variability in microbiology data, reliability and reproducibility depend on the use of accepted methods and adherence to good laboratory practices.

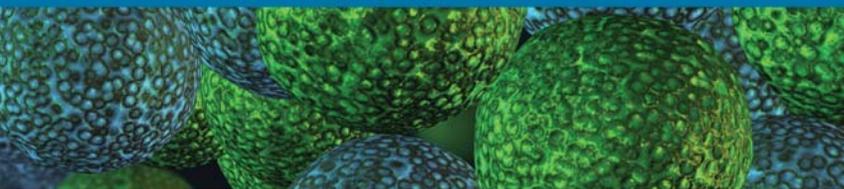
Media control is central to microbiology and plays a large role in the design of different compendial tests (Cundell 2002). The control and qualification of test strains, equipment, and so on importantly limit variability in test results (Sutton 2010c). Taken as a whole, this chapter can serve both as the backbone of the laboratory SOP system (Sutton 2010d) and as a useful guide for auditing and qualification of contract laboratories (Sutton 2011a).

The final section in the chapter discusses interpretation of data. This section is important for two reasons: First, it emphasizes accurate and consistent operations in the lab, extending to explicit rules for handling numbers (see the useful discussion of significant digits and rounding by Torbeck 2004). Second, it provides a good introduction to investigations in the microbiology lab—an important topic in today’s regulatory climate (Sutton 2011b).

After several years of use, this chapter was recommended for revision in several specific areas (USP 2009h). New topics included a discussion of sample handling, determination of media incubation times, and laboratory resources. Sample handling arose from concern that analysts are, after all, testing living organisms that respond to external stimuli, and analysts must take care that the sample finally tested is similar to the fresh sample. The larger question of media incubation times has been a difficult one for the industry: when instructions say to incubate for 3 days does this mean 72 hours, 64–80 hours, or something else? The third new section directly addresses the concern that the microbiology laboratory must be provided sufficient resources in terms of labor and budget to meet its obligations. This section also provides some suggestions for metrics to determine the efficiency of the lab—metrics useful not only for staff qualification but also for lean laboratory exercises.

Several sections were expanded as well. For example, many changes were introduced into the section about Training because of the importance of that section. Emphasis was placed on continuing training for all members of the lab (for a helpful discussion of microbiological training opportunities see Nicoll 2005). In addition, the chapter included an admonition about the need for scientifically competent leadership of the lab, bringing the section in line with the CDC Biosafety manual’s recommendation for laboratory leadership in a Biosafety Risk Level 2 laboratory (CDC 2007). The Data Interpretation

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	BioTyper MALDI-TOF	72%	2%	\$30*

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section was extensively expanded with discussions of laboratory investigations and formal introduction of the term of Microbiological Data Deviation into the regulatory lexicon to describe the subject of these laboratory investigations.

This chapter was published in final form in Supplement 2 of USP 35 (2010e).

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## <1208> Sterility Testing—Validation of Isolator Systems

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The revision proposed in 2004 to this draft chapter replaced 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 brought the chapter more closely into alignment with ISO standards 14644-1, -2, -3, and -7 (USP 2004b). This chapter became official in the Second Supplement to USP 30 (2007i).

The validation of isolator systems in preparation of sterility testing has become more important as the use in this application has become more prevalent. The USP MSA committee is not the only body that has addressed this topic—the Pharmaceutical Inspectorate Convention Scheme (PIC/S) also has published useful guidance (PIC/S 2007). In addition, Byrne reviewed the topic from the user’s perspective (Byrne 2005), and Reich pointed out considerations that may confound the validation and described how to recognize them (Reich 2005).

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## <1211> Sterilization and Sterility Assurance of Compendial Articles

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This chapter had been neglected since it was created during the late 1980s and is in need of revision. The first serious revision to the chapter in almost 25 years appeared in the September–October 2004 PF (2004c). This proposed In-Process Revision provided several updates to the chapter. The lethality value (F<sub>0</sub>) was introduced in the chapter. The revision incorporated updated taxonomic terms for biological indicator species were incorporated and discussed differences between biological indicator usages for moist-heat vs. gas sterilization. In addition, the sections that cover various modes of sterilization have been revised and updated, and a new section includes vapor-phase hydrogen peroxide sterilization. Finally, the EC deleted the discussion pertaining to Stage 2 of sterilization testing in order to reflect the currently official chapter Sterility Tests <71> provisions that have been in effect since 1998 (Sutton 2001).

This proposal was never finalized, and in 2009 a second proposed revision was put forward as an interim step to eliminate reference to the discussion of the defunct nonharmonized sterility tests with first- and second-stage tests and to the older radiation sterilization guidance, guiding the reader instead to relevant ISO standards (USP 2009i). This interim text was made official in the Second Supplement to USP 33 (reissue) (USP 2010f).

The revisions of <1211> have been difficult to implement, not because of the paucity of information but perhaps because of the depth of the chapter and the passion of the committee members on the subject. As an example, Table 3 shows a listing of recent articles published by members of the MSA committee regarding different aspects of this topic. The current plan for this chapter is to break it down into smaller chapters based on method of sterilization (chemical sterilization, dry-heat depyrogenation, gas-sterilization filtration, dry-heat sterilization, radiation sterilization, steam sterilization, and vapor sterilization). This plan is reviewed in Sutton (2009c).

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## <1222> Terminally Sterilized Pharmaceutical Products—Parametric Release

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This chapter originally appeared in the First Supplement to USP 2004 (USP 2004d). However, several minor revisions were proposed in 2004 (USP 2004e). These revisions included clarifying the use of the sterility assurance level with aseptic processing and revising the discussion of the number of spores required for the evaluation of a sterilization process. These changes were adopted, and the finalized chapter appeared in the Second Supplement to USP 30 (USP 2007j). A helpful review of ways this chapter can guide a regulatory strategy for parametric release was published in 2005 and stressed the need for parametric release of product to be considered only as a component in a comprehensive and controlled production process (Tirumalai and Porter, 2005).

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## <1223> Validation of Alternative Microbiological Methods

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This chapter began in the 2000–2005 revision cycle and underwent several important changes before the draft version published in 2005. This version focused on qualitative and quantitative aspects of microbiological method validation, and the earlier section on microbial identification was removed (Sutton and Cundell 2004). This revised version of the chapter was finalized in Supplement 2 of USP 29 (USP 2006g).

This topic continues to be discussed by the committee and USP as a whole. One indication of this interest is the large number of Stimuli articles that address different aspects of the statistics and applications of rapid microbiological methods (see below). Another is the large number of articles published by committee members (Table 4), which reflects the high degree of interest in this topic in the industry.

## Stimuli to the Revision Process

Pharmacopeial Forum provides a mechanism for interested parties to publish scientific articles of interest to compendial scientists. During the 2000–2005 revision cycle several articles were either of general interest to the microbiological community or addressed topics directly overseen by MSA EC.

*USP Project Team 7. 2005. Microbial Testing for Orally Inhaled and Nasal Drug Products. Pharmacopeial Forum. 31(4):1258–1261.*

The Aerosols Expert Committee proposes adding the following text to a future version of General Chapter (601) Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers to provide microbiological sampling methods and accompanying specifications for orally inhaled and nasal drug products (OINDP) that are not required to be sterile. Note that Aqueous OINDP (e.g., single-dose inhalation solutions or aqueous solutions for inhalation) are required to be sterile, and these products are not addressed here except that certain general principles concerning aseptic sampling, as described below, may also apply to sterile OINDP during opening and testing of finished products. These procedures have been developed with the assistance of USP's Project Team 7.

*Bao, Y, et al. 2010. A Rapid Capillary Electrophoresis Method for the Detection of Microbial Contamination: An Alternative Approach for Sterility Testing. Pharmacopeial Forum. 36(6):1798–1805.*

This Stimuli article discusses capillary electrophoresis (CE) methodology for sterility testing as a possible alternative to the traditional direct inoculation method and more recent molecular techniques involving PCR/DNA testing and antibody-based methods. Topics discussed include basic CE theory, CE characterization of bacteria and fungi, CE sterility testing method development, and procedures based on the proposed method. Sample preparation and/or preconcentration procedures for CE analyses are considered as well. Finally, the article briefly demonstrates use of CE to analyze actual consumer products.

*Hauck W, et al. 2009. Acceptable, Equivalent, or Better: Approaches for Alternatives to Official Compendial Procedures. Pharmacopeial Forum. 35(3):772–778.*

The General Notices in US Pharmacopeia (USP) permit analysts to use acceptable (suitable) alternatives to an official procedure. In

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a revision that will be effective May 1, 2009, the General Notices will require that the alternative procedure be demonstrated to give results that are equivalent to or better than those obtained by the official procedure. This Stimuli article discusses approaches for determining equivalent or better procedures. The concepts and tests discussed in this paper may become one or more USP General Chapters.

*Marques, MRC, et al. 2009. Liquid-filled Gelatin Capsules. Pharmacopeial Forum. 35(4): 1029–1041.*

This Stimuli article provides an overview of the manufacturing, characteristics, and in vitro performance evaluation of liquid-filled gelatin capsules. The intent of the article is to initiate discussion, to solicit public comments, and to invite participation of interested parties in the efforts of the USP Biopharmaceutics Expert Committee in either updating USP General Chapter Dissolution <711> or creating a new general chapter that will address the particularities and special approaches required to develop and carry out in vitro performance evaluations of liquid-filled gelatin capsules.

*Hauck, W, Koch W, et al. 2008. Making Sense of Trueness, Precision, Accuracy, and Uncertainty. Pharmacopeial Forum. 34(3):838–842.*

Understanding terminology is important in order for scientists to be able to communicate with each another. This Stimuli article thus reviews the terms accuracy and precision, along with the related terms, trueness and uncertainty. The goals are to be clear where there is agreement between the usage in USP's compendia and in the international metrological community and where there is not and to make recommendations regarding use of these terms in USP and its compendia.

*Tan, CY. 2005. RSD and Other Variability Measures of the Lognormal Distribution. Pharmacopeial Forum. 31(2):653–655.*

This Stimuli article reviews relative standard deviation (RSD) in the context of the lognormal distribution. The article discusses the advantage of RSD compared to standard deviation. Other variability measures that offer similar advantage are discussed and compared.

*Garfinkle BD, et al. 2005. The Development of Chapter <1235> Vaccines and Vaccine Test Methods. Pharmacopeial Forum. 31(5): 1533–1538.*

This Stimuli article provides introductory sections and the intended scope of a comprehensive USP general information chapter on vaccines and vaccine test methods currently under development by the USP Vaccines, Virology, and Immunology Expert Committee. The authors publish this information as a Stimuli article with the intent of initiating discussion and public comment. Volunteers

to develop these sections and/or review completed sections are welcome.

*Lockhart H, Selke S, et al. 2006. The Role of Container–Closure Systems in Stability Testing for Climate Zone IV. Pharmacopeial Forum. 32(2):688–694.*

The authors suggest that designation of long-term stability test conditions must include the effect of barrier packaging on the atmosphere inside the package. In December 2004, the Association of South East Asian Nations (ASEAN) proposed to the World Health Organization (WHO) that the standard for long-term stability testing for markets in Climate Zone IV be changed from 30°C, 65% relative humidity (RH) to 30°C, 75% RH. The argument for this proposal was based on the effect of absolute humidity on drug products. It did not take into account the effect of barrier packaging on control of the absolute humidity inside the package. The authors stipulate that selection of stability test conditions must include consideration of package permeation, storage temperature, and the permeation driving force, i.e., partial pressure for water vapor. The authors show that for challenge of the packaged drug product, relative humidities lower than 75% at 30°C would adequately represent the ASEAN countries' Climate Zone average condition of 27°C, 79% RH. Laboratory testing is advised.

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## Conclusions

This has been a busy revision cycle for the MSA EC. Table 1 provides a summary of the publications for each chapter. This table clearly shows that even after a chapter is "finalized" and published in a USP Supplement, opportunities remain to improve it or to change aspects of the test. USP is committed to continuous revision and improvement and looks for input from the field. Indeed, this input is critical to the success not only of USP but of the industry as a whole because we all suffer when chapters are finalized when they are not sufficiently broad in application or scope or present technical challenges to execution beyond the current state of the industry.

The upcoming revision cycle promises to bring continued change. New informational chapters about quality issues in the microbiology lab promise to continue USP leadership in this area, and the expected wholesale revisions of informational chapters regarding clean rooms, bioburden control, monitoring for nonsterile product manufacture, and sterility assurance will help to ensure a safe product supply. Many comments come in to USP regarding new information that workers would like to see, and some of these are under development. The need for change has not ended with the 2005–2010 revision cycle.

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MALDI-TOF, invented by Shimadzu and awarded the Nobel prize in 2002, is now optimized for routine use by co development with bioMérieux.



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## Author Biographies

**Dr. Scott Sutton** earned his B.S. in Genetics from the University of California at Davis, and his Masters and Ph.D. in Microbiology from the University of Rochester (Rochester, NY). After an NIH postdoctoral fellowship at the Medical College of Virginia (Richmond, VA), he went to work for Bausch and Lomb (Rochester, NY) until 1994 when he accepted a position at Alcon Laboratories (Fort Worth, TX). Dr. Sutton left Alcon Laboratories in 2004 as a Director in the R&D division to accept a position as Pharma Consultant (Microbiology) with Vectech Pharmaceutical Consultants, Inc which he left in 2009 as Sr. Director, Microbiology Services.

Scott Sutton is the Principal of Microbiology Network, Inc, a company he started in 1996 as a means to encourage training and communications within the microbiological community. He is a recognized consultant and trainer with emphasis in GMP, investigations, Environmental Monitoring and contamination control (both Aseptic manufacturing and non-sterile production facilities) and microbiology laboratory audits and operations. The Microbiology Network supplies consulting, training, webinars and Email discussion groups (PMFList, PSDGList and C-CEList) Dr. Sutton is an active author and speaker for the industry, supports PDA and has served with the USP Analytical Microbiology Committee of Experts since 1993.

**Dr. Tirumalai** has been at USP since 2003 and is currently a Senior Scientist in the Documentary Standards/General Chapters Division. He is the Staff Liaison to the USP Expert Committees on Microbiology and Sterility Assurance, and General Toxicology and Medical Device Biocompatibility. He works with industry, academia, and government as the USP Expert Committee liaison in the development and revision of General Chapters and Monographs in these areas.

Dr. Tirumalai's prior industry experience encompasses process and product research and development, transfer, and product manufacturing. His postdoctoral work included studies on HIV and MuLV reverse transcriptase and bacteriophage lambda integrase. He has authored several publications and review articles