

The International Harmonization of the Compendial Microbial Limits Tests— A Cautionary Tale of Compendial Participation

Scott Sutton



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Welcome to “Microbiology Topics.” This column discusses various topics in microbiology of practical use in validation and compliance. We intend this column to be a useful resource for daily work applications.

Considerations associated with microbiology are part of every pharmaceutical and medical device product. Microbiology and applications thereof are fundamental to daily operations, quality assurance and control, compliance, validation, and so on—literally all aspects of manufacturing and compliance. These topics require knowledge and understanding of basic technical principles for sound decisions in everyday work situations.

Microbiology principles are complex. New technology is evolving, and the field is becoming increasingly specialized. These considerations are relevant to a wide range of circumstances including synthesis of biotech products, sterile product and device processing, microbial and environmental monitoring of facilities and utilities, sterilization processes, microbial analytical methods, oral and topical product manufacturing, and so on—a diverse range of applications. Further, the language of microbiology associated with the aforementioned may be esoteric and intimidating. These considerations make discussion of relevant microbiology topics a challenging task. This column will address microbiology topics with these difficulties in mind. It is our challenge to present microbiology topics clearly and using understandable language to provide useful information for daily work applications.

Reader comments, questions, and suggestions are needed to help us fulfill our objective for this column. Case studies from readers are also most welcome. Please send your comments and suggestions to column coordinator Scott Sutton at scott.sutton@microbiol.org or journal coordinating editor Susan Haigney at shaigney@advanstar.com.

KEY POINTS

This article discusses the finalized harmonization of the Microbial Limits Tests. Changes to the respective tests and the ramifications of these changes are addressed. Specific key points discussed in this article include the following:

- The international harmonization of the *United States Pharmacopeia* (USP), *Japanese Pharmacopeia* (JP), and the *European Pharmacopoeia* (EP, *Pharm Eur*) Microbial Limits Tests is now finalized

- *USP* chapters involved in this harmonization include <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests; <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms; and <1111> Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances For Pharmaceutical Use
- Changes to the bioburden test group (*USP* <61>). There are significant changes in test method details including organism type, preparation methods, and other requirements. The demonstration of media growth promotion and selective and differential properties is significantly more detailed in the harmonized procedures.
- Changes to the specified organisms tests (*USP* <62>). The old test used four organisms; the new tests describe tests for seven organisms (or types). There also are changes in broths (media), inoculum concentrations, temperature of incubation, and dilutions for these tests.
- Significant changes in *USP* <1111> involving identification of “other” organisms were implemented. *USP* <1111> and CFR 211 have consistent requirements regarding absence of objectionable organisms
- Industry responses to *Pharmacopeial Forum* (*PF*) notices of the above changes were not submitted to *USP* before signoff of the international harmonization agreement. Consideration of comments from industry necessitated postponing the targeted implementation date to allow industry time to implement the changes.
- When monograph or section changes are published in *PF*, industry should closely evaluate all proposed changes and respond with questions or concerns in a timely manner. Timeliness of responses is especially important for changes in chapters under consideration for international harmonization.

INTRODUCTION

The international harmonization of chapters in the *United States Pharmacopeia* (*USP*), *Japanese Pharmaco-*

peia (*JP*), and the *European Pharmacopoeia* (*EP*, *Pharm Eur*) regarding Microbial Limits Tests is now finalized. Discussions began in the 1990s to determine the best approach to harmonization of the Microbial Limits Tests (1,2,3). The initial proposal to split the then-current *USP* chapter <61> into two chapters (“bioburden” and “absence of specified organisms”) drew comment, and on the basis of the comments, work proceeded (4). Preliminary drafts of harmonization proposals were published in 2001 (5, 6, 7). These drafts also drew a great deal of comment, and in a meeting of the Pharmacopeial Discussion Group (PDG) significant changes were introduced and prepared for publication and public comment (8).

The near-final drafts of these chapters were published in 2003 as part of the harmonization process (9,10,11) to minimal comment. These drafts, with minor revision, were approved in 2005 by the three participating pharmacopeia. The final drafts were published in 2006 (12,13,14), originally with an implementation date of May 1, 2007, which was later changed to May 1 of 2009 (15). Minor changes were made in 2007 and 2008 before implementation of the now-current versions (16).

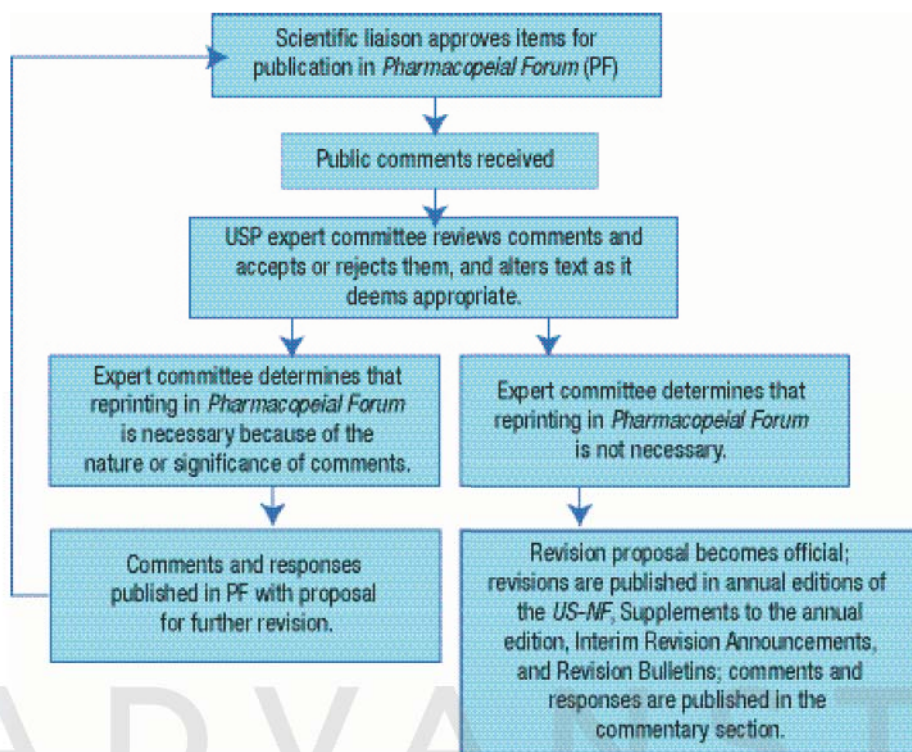
While this sequence of events is of some interest, it really does not tell the entire story—a story that can serve as a cautionary tale of the need for close cooperation between *USP* and the interested industrial community. Further, this story demonstrates the potential impact that revisions in *USP* may exert on microbiology laboratory operations and compendial compliance. Before going on, it must be noted that the author of this piece is writing as an individual with personal knowledge of the events, but is not a representative of *USP* nor are the opinions expressed in this article those of the United States Pharmacopeia.

THE IMPORTANCE OF READING *PF*

The purpose of the journal *Pharmacopeial Forum* (*PF*) is to publicize upcoming changes in the compendium and in the *National Formulary* (*NF*) before they come into effect. This is intended to provide a forum for the industry and other interested parties to comment on the drafts before they become official. Proposed drafts to the Microbial Limits Tests were published in 2001

Figure:

Public review and comment process for *USP-NF* standards development (adapted from USP website on September 13, 2009).



(to significant comment from the field) and amended versions were published in 2003. There was little if any comment to these 2003 drafts. This came as a surprise to the USP Expert Committee, which expected chapters that had been modified significantly from the previously published versions to generate significant input.

The public review process for USP is detailed in the Figure. As is apparent, public comment is vital to the process and the interested reader is referred to several excellent articles on the USP revision process for further discussion on this topic (17,18,19,20). Experience has taught that input from the field is rarely if ever congratulatory in nature. However, the input received in response to the publication of a draft proposal is frequently quite useful in pointing out errors, inconsistencies, technical difficulties, and areas of disagreement. This is expected and welcomed as the input broadens and strengthens the draft chapter. In the absence of this comment from the field, the committee is forced to conclude that the proposal is acceptable.

In the case of the Microbial Limits Tests in the 2003-2006 timeframe, there was little comment, and so the final draft versions were published in 2006 containing few changes with an effective date of mid-2007. Then the comment came in—too late. As the chapters in question were now signed off as internationally harmonized, the USP no longer had the ability to change the text of the approved chapters without getting international agreement. However, because of the volume (and passion) of the communications, something had to be done. The decision was made to postpone implementation to allow industry time to implement the changes. As stated on the USP website in 2006 (21):

“...microbiological quality *USP-NF* General Chapters has been postponed until May 1, 2009.

- <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests
- <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms
- <1111> Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical

Preparations and Substances For Pharmaceutical Use

“These *USP–NF* General Chapters were originally scheduled to be effective on August 1, 2007. These postponements are made in response to requests received from the user community to allow further time to implement the new methods, and to harmonize with the implementation schedules of the European and Japanese Pharmacopoeias.

“The currently official *USP–NF* General Chapters <61> Microbial Limit Tests and <1111> Microbiological Attributes of Nonsterile Pharmaceutical Products will remain effective until May 1, 2009.”

This was an important concession, as the new tests, apparently unnoticed by industry, were different in significant details from the previous chapters.

Differences Between the New and Old Tests

The Microbial Limits Tests, as the name implies, are actually a collection of tests and specifications. The major divisions are along chapter organization. *USP* chapter <61> (*Pharm. Eur.* 2.6.12) addresses bio-burden testing, both Total Aerobic Microbial Count (TAMC) and Total Yeast and Mold Count (TYMC), while *USP* <62> (*Pharm. Eur.* 2.6.13) describes tests for the “absence of” seven different specified organisms. The specifications these tests support are described in at least one monograph of at least one of the three regional pharmacopeia. Finally, *USP* chapter <1111> (*Pharm. Eur.* 5.1.4) is an informational chapter to assist in setting microbial quality standards for those products and raw materials not covered by monograph in the *National Formulary* (NF).

Specified Organisms Tests and Compliance Considerations

The first tests examined in this article are the tests for specified organisms (*USP* <62>). There are now seven separate tests described in the harmonized chapter, an increase from the four that existed previously in *USP*. We clearly will need to perform work to demonstrate method suitability for any test of the “absence of” a novel index organism. The following are three of these newly specified organisms for *USP*:

- Bile tolerant gram-negative bacteria (replaced the EP procedure “Test for Enterobacteria and Certain Other Gram-Negative Bacteria”)
- *Clostridia*
- *Candida albicans*.

If a company has a regulatory commitment to test “as per *USP* Microbial Limits Tests,” this might be a difficult position. Can product release occur under this commitment without meeting it fully? Does the company need to add testing for these organisms even though it has been safely releasing product for years without these specifications? These are fundamental compliance questions.

This situation might not be as obvious for the other tests in the “absence of specified organisms” battery. The “absence of specified organisms” tests continues to provide procedures for demonstration of the absence of:

- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Salmonella spp*
- *Escherichia coli*.

For example, do the method suitability tests for “absence of” these organisms have to be repeated? The first step of both old and new methods is an enrichment to encourage growth of low numbers of the specified organism, followed by subculture of the microbial growth suspensions on selective and/or differential media. The intent of the incubation on selective media is to depress the growth of microorganisms, allowing a selective advantage to the more resistant “specified” microorganisms. The differential media was formulated to distinguish the colony morphology of the “specified” organisms from others, allowing visual identification. As stated previously, this fundamental approach is unchanged in the harmonized versions. However, the enrichment broth has changed in some of the new tests and the selective and differential scheme has changed in most. While it might be possible to argue that if the old test worked the new test should also, this is a high-risk approach. A major problem with this assertion is that sooner or later it will be challenged and data will be required as part of the justification.

Should a company decide to justify existing method suitability studies as meeting current requirements there is at least one more consideration. The new tests have far more detail as to challenge organism (i.e., identity, growth conditions, and inoculum size) than were in the “Preparatory Testing” sections of the previous USP tests. This section in the previous USP chapter <61> described the method suitability study designs for both the enumeration and the “absence of” tests. The inoculum concentration especially might be incompatible between the two versions, as the previous preparation instructions were to add “1 mL of not less than 10^3 dilution of a 24-hour broth culture of the microorganism to the first dilution...” As this 24-hour broth culture will consist of approximately 10^7 - 10^8 CFU/mL, the inoculum is therefore about 10^4 - 10^5 CFU. The current version specifies not more than 100 CFU total. In addition, the inocula are now required to be used fresh where before there were no specifications of this type in the USP prior to the harmonized method.

Bioburden Tests and Compliance Considerations

The second Microbial Limits test group is the bioburden test (USP <61>), which contains two separate tests: Determination of TAMC and determination of TYMC. While this test is not significantly changed from the portion of the previous microbial limit test that dealt with bioburden testing, the test procedure also includes a significant increase in detail. The method suitability procedure can serve as an example here too. In this study, the inoculum has changed in regards both to type (i.e., differences in organisms) and detail (i.e., described to national stock culture collection number). Additionally, the preparation has changed. The older test described the use of 1 mL of a 10^{-2} dilution of an overnight culture (approximately 10^5 - 10^6 CFU/mL from an initial concentration of 10^7 to 10^8 CFU/mL) while the harmonized test set the inoculum level at <100 CFU. This is a significant difference from the previous method. The harmonized test describes the growth of the challenge organisms in great detail, how to harvest and re-suspend the cells, requiring the use of a fresh inoculum preparation and providing that the inoculum should not add more than 1% to the volume of the sample. This is all new to USP. In addition,

like the test for “absence of specified organisms,” media growth promotion requirements are far more stringent in the harmonized test than in the previous version. Finally, as this is a quantitative test, the harmonized procedure provides acceptance criteria (at least 50% recovery of inoculum in the presence of the test sample) while the previous USP version was silent on this issue.

A final consideration in comparing the old and new Microbial Limits Tests involves the media used in the testing. If you claim that the test is “performed as per USP,” that of course means that you will follow all aspects, not just some. The demonstration of growth promotion and selective and differential properties is significantly more detailed in the harmonized procedures. The previous USP version of the Microbial Limits Tests provided minimal direction as to media quality control and so it is extremely unlikely that media used in previous studies meets current requirements.

USP <1111>, Section “Other Organisms”

One of the interesting, if little discussed, aspects of the harmonized Microbial Limits Tests is the now marked similarity between USP <1111> (*Pharm Eur* 5.1.4) and the 21 CFR 211 requirements for absence of “objectionable organisms.”

Before the introduction of the harmonized chapter, USP required testing for the presence of specified organisms such as Salmonella, E. coli, and others. These organisms are specified in article monographs. The harmonized informational chapter (USP <1111>, *Pharm. Eur* 5.1.4) discusses the need to evaluate “other” organisms and is consistent with US Food and Drug Administration concerns regarding non-sterile products and “objectionable organisms.” This is a big shift from the previous text in this chapter, but makes sense from a compliance standpoint. After all, what are the microbiology quality requirements for non-sterile products? Going back to the General Notices and Requirements section 3.10.10, USP 32 (2009) states, “The applicable USP or NF standard applies to any article marketed in the United States that (1) is recognized in the compendium and (2) is intended or labeled for use as a drug or as an ingredient of a drug.” Section 3.10.20 extends this to medical devices if they are labeled as complying with USP. This seems to be stating plainly that the USP

chapters labeled under 1000 are required only for those products for which there are monographs in the *National Formulary*, or those medical devices labeled “USP.” For most products then, chapters <61> and <62> are not a USP requirement. Even for those monograph products the standard seems to be “if tested shall comply” as presented in section 3.10 Applicability of Standards:

“... The manufacturer’s specifications, and good manufacturing practices generally, are developed and followed to ensure that the article will comply with compendial standards until its expiration date, when stored as directed. Thus, any official article tested as directed in the relevant monograph shall comply” (General Notices 3.10 second paragraph *USP* 2009).

What about good manufacturing practice (GMP)? One aspect is the recognition of USP by 21 CFR 211.194, which states in 211.194 Laboratory Records (a)(2):

“A statement of each method used in the testing of the sample. The statement shall indicate the location of data that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability as applied to the product tested. (If the method employed is in the current revision of the *United States Pharmacopeia*, *National Formulary*, Association of Official Analytical Chemists, *Book of Methods*, or in other recognized standard references, or is detailed in an approved new drug application and the referenced method is not modified, a statement indicating the method and reference will suffice). The suitability of all testing methods used shall be verified under actual conditions of use.”

So the perception is that if you follow the USP you are following GMPs. While this may be true in many cases, we also have to consider the issue of “objectionable organisms.”

The phrase (or concept) of “objectionable organisms” appears three times in 21 CFR 211:

- **21 CFR 211.84(d)(6).** “Each lot of a component, drug product container, or closure with potential for microbiological contamination that is objectionable in view of its intended use shall be subjected to microbiological tests before use.” The recent changes in GMP effective December 8, 2008 included changing the phrase “that is liable to” for “with potential for” in this section.

- **21 CFR 211.113(a).** “Appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, shall be established and followed.”
- **21 CFR 211.165(b).** “There shall be appropriate laboratory testing, as necessary, of each batch of drug product required to be free of objectionable microorganisms.”

It has been suggested that the Microbial Limits Tests will meet these requirements and demonstrate the absence of objectionable organisms (3). However, this position is not supported by FDA (22) nor by USP (23). The harmonized informational chapter *USP* <1111> (*Pharm Eur*. 5.1.4) now includes the explicit recommendation (16):

“In addition to the microorganisms listed in Table I, the significance of other microorganisms recovered should be evaluated in terms of the following:

- The use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract)
 - The nature of the product: Does the product support growth?
 - Does it have adequate antimicrobial preservation?
 - The method of application
 - The intended recipient: risk may differ for neonates, infants, the debilitated
 - Use of immunosuppressive agents, corticosteroids
 - The presence of disease, wounds, organ damage.
- “Where warranted, a risk-based assessment of the relevant factors is conducted by personnel with specialized training in microbiology and in the interpretation of microbiological data. For raw materials, the assessment takes account of the processing to which the product is subjected, the current technology of testing, and the availability of materials of the desired quality.”

This certainly sounds like an evaluation for objectionable organisms by a competent, trained, professional microbiologist (24).

Bringing this academic discussion into the real world, FDA has a solid track record of enforcing this requirement. A recent review of recalls from 1998 through

2006 (25) examined 134 recalls listed on the FDA website for this time period. Of these, only 14 were due to organisms listed in the Microbial Limits Tests. The others were “objectionable” but not “specified.” FDA will enforce a requirement for “absence of objectionables” in non-sterile marketed products irrespective of the regulatory commitment made by the company for finished product release testing of those batches.

This might actually be the biggest change to the tests that no one really is discussing, and it was added to the drafts without a comment and continues to be roundly overlooked.

This provides an interesting philosophical question. Is the Microbial Limits Test really suitable as a product release test for non-sterile finished dosage forms? Is it an appropriate quality control (QC) test for product release when it fails to demonstrate the key specification, in this case the absence of “objectionable organisms”? It was not, after all, designed as a QC test, but rather a referee test to demonstrate compliance with specific monograph requirements.

WHERE DOES THIS LEAVE US?

I would urge the close evaluation of all proposed changes in USP chapters and immediate, reasoned, and complete responses to USP on any questions or concerns. For those who believe little is left to contribute, the following is a list of drafts to microbiology-related chapters that have appeared in the first five (of six) issues of *PF* during 2009:

- <1> Injections. *Pharm Forum* 35(5): 1214-1217
- <41> Weights and Balances. *Pharm Forum* 35(2):331 - 334
- <63> Mycoplasma Tests. *Pharm Forum* 35(1): 146 - 152
- <645> Water Conductivity. *Pharm Forum* 35(5): 1226-1228
- <1113> Microbial Identification. *Pharm Forum* 35(1) :167 - 175
- <1117> Microbiological Best Laboratory Practices. *Pharm Forum* 35(4): 945-951
- <1211> Sterilization and Sterility Assurance of Compendial Articles. *Pharm Forum* 35(4): 952-960
- <1231> Water for Pharmaceutical Purposes. *Pharm Forum* 35(5): 1310-1319
- <1235> Vaccines for Human Use - General Considerations. *Pharm Forum* 35(4): 960-983
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Without the full participation of industry the revision process of USP becomes isolated from stakeholders. Much can be learned, and should be learned, from the unfortunate circumstances surrounding the harmonization and implementation of the Microbial Limits Tests. We have a unique opportunity to play a role in the development of compendial standards through the USP revision process. All that is required is our desire to do so and the will to act.

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ARTICLE ACRONYM LISTING

| | |
|-------------|-------------------------------|
| EP | European Pharmacopoeia |
| JP | Japanese Pharmacopoeia |
| NF | National Formulary |
| QC | Quality Control |
| TAMC | Total Aerobic Microbial Count |
| TYMC | Total Yeast and Mold Count |
| USP | United States Pharmacopoeia |

ABOUT THE AUTHOR

Dr. Scott Sutton earned his Masters and Ph.D. in microbiology from the University of Rochester (NY). After a NIH postdoctoral fellowship, he worked for Bausch and Lomb until 1994 when he went to Alcon Laboratories. In 2004, Dr. Sutton accepted a position with Vectech Pharmaceutical Consultants. He has worked with the USP Microbiology Committee of Experts since 1993, serving as vice-chair since 2000. Dr. Sutton also operates an information source on the Internet, The Microbiology Network (www.microbiol.org), which provides services to microbiology-related user's groups and supports the PMFList, a microbiology e-mail list (www.microbiol.org/pmflist), and the PSDG e-mail list (pharmaceutical stability topics) (www.microbiol.org.psdglist). Dr. Sutton is a consultant in contamination control, microbiology, aseptic manufacturing, and rapid microbiology methods. Dr. Sutton can be reached at scott.sutton@microbiol.org.