

Does International Harmonization of the USP Microbial Limits Tests Require Re-Validation of Finished Product Tests?

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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 addresses microbiology topics with these difficulties in mind. It is our challenge to present microbiology topics clearly, 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 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 include the following:

- The final versions change the requirement for validation to "verification of suitability of the method"
- The Microbial Limits Tests are a collection of tests and specifications. *United States Pharmacopeia (USP)-National Formulary (NF) chapter <61>* looks to bioburden testing (total aerobic microbial

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count [TAMC] and total yeast and mold count [TYMC]) while USP <62> describes tests for the “absence of” seven different specified organisms. USP <1111> is an informational chapter for setting microbial quality standards for non-NF materials.

- USP <61> describes bioburden testing (TAMC and TYMC). Specific details of these tests have been changed including inoculum broth, preparation, dilution requirements, and growth promotion
- USP <62> describes seven separate tests for the following: Bile tolerant gram-negative bacteria, *Clostridia*, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella spp*, and *Escherichia coli*. In addition to new organisms, there are changes in media requirements.
- Product risk analysis including product use and route of administration, growth potential, preservation, and other considerations is recommended in USP <1111>. There have been product recalls due to the presence of objectionable organisms.
- Re-validation of existing tests to current harmonized standards and level of detail using a matrix approach is discussed
- The fundamental shortcomings of these tests in regards to the current good manufacturing practice (CGMP) requirements for “absence of objectionable organisms” is discussed.

INTRODUCTION

The international harmonization of the Microbial Limits Tests in the *United States Pharmacopeia (USP)*, *Japanese Pharmacopeia (JP)*, and the *European Pharmacopoeia (EP, Pharm Eur)* was finalized in 2009. The near-final drafts of these chapters were published in 2003 as part of the harmonization process (1,2,3) to minimal comment. These drafts, with minor revision, were approved in 2005 by the three participating pharmacopeia. The final drafts were published in 2006 (4,5,6), originally with an implementation date of May 1, 2007 (USA), which was later changed to May 1 of 2009 (7). Since then, minor changes were made in 2007 and 2008 before implementation of the final draft versions (8).

The final draft versions have a change that is of interest to the general topic of validation. References in the text of both USP <61> and <62> to method validation were replaced by “verification of suitability of the method” (1,2). This was done at the request of USP for the harmonized chapters, because the mandatory chapters of USP are provided to test products

to monograph requirements (see *USP General Notices: 2.10 Official Text*) and are validated for this purpose by definition. Validating a validated method seems redundant. If we wish to use an alternate method to test an official article (Section 2.20) to monograph specification, the non-USP method must be validated. The actual question we are interested in answering is whether or not the method is suitable for testing a particular product. The harmonized methods include somewhat detailed instructions on this procedure for each test. These method suitability studies are the focus of this article.

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> looks to bioburden testing (both TAMC and TYMC) while <62> 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 <1111> 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*.

Tests For Specified Organisms

The first tests we can look at are the tests for specified organisms (USP <62>). There are now seven separate tests described in the harmonized chapter, up from the four that existed previously in the 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 you have a regulatory commitment to test “as per USP Microbial Limits Tests” you might be in a difficult position. Can you release product under this commitment without meeting it fully? Do you need to add testing for these organisms even though you have safely been releasing product for years without these specifications? This is a fundamental compliance question that you must answer as a company. If

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you determine that you must comply fully, additional work will be in order at least for these organisms.

The question of re-validation (or demonstration of method suitability) might not be as obvious for the other tests in the “absence of specified organisms” battery of tests. The “absence of specified organisms” tests continue to provide procedures for demonstration of the absence of the following:

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

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/differential scheme has changed in most. While it might be possible to argue that if the old test worked the new should also, this is a high-risk approach. This assertion is likely to be challenged at some point and data will be required as part of the justification.

Should you 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 <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. In other words, if you were in compliance with the old tests, those same studies cannot be in total compliance with the harmonized tests. Whether this difference is significant to the outcome of the test is for the company to determine and justify their decision (although a 1000-fold to 10,000-fold difference in the inoculum seems rather large to ignore).

Bioburden Test

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, this test also has enjoyed a significant increase in detail. The inoculum has changed as to both type (i.e., differences in organisms) and detail (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 sets 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) where the previous USP version was silent on this issue.

Media Quality Control Requirements

A final major consideration in the decision of whether or not to repeat method suitability determinations 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 version of the Microbial Limits Tests for specified organisms barely discusses media quality control at all, and so it is extremely unlikely that media used in existing method suitability studies meet current requirements. Again, any claim that an existing method suitability study is adequate must be able to withstand challenge in an audit.

Overviews of the method suitability study designs are presented in Figure 1 (older requirements of the regional [US] pharmacopeia) and Figure 2 (the current, harmonized test requirements).

In the end, it is possible to use existing method suitability studies for the new tests if they are done according to the detailed instructions present in the harmonized USP <61> and <62> (*Pharm Eur* chapters 2.6.12 and 2.6.13) and the tests have sufficiently detailed documentation to show this. If either of these conditions is not true (which is the overwhelmingly likely situation), the author's recommendation is to repeat the method suitability studies to current standards if you wish to claim compliance with the USP method.

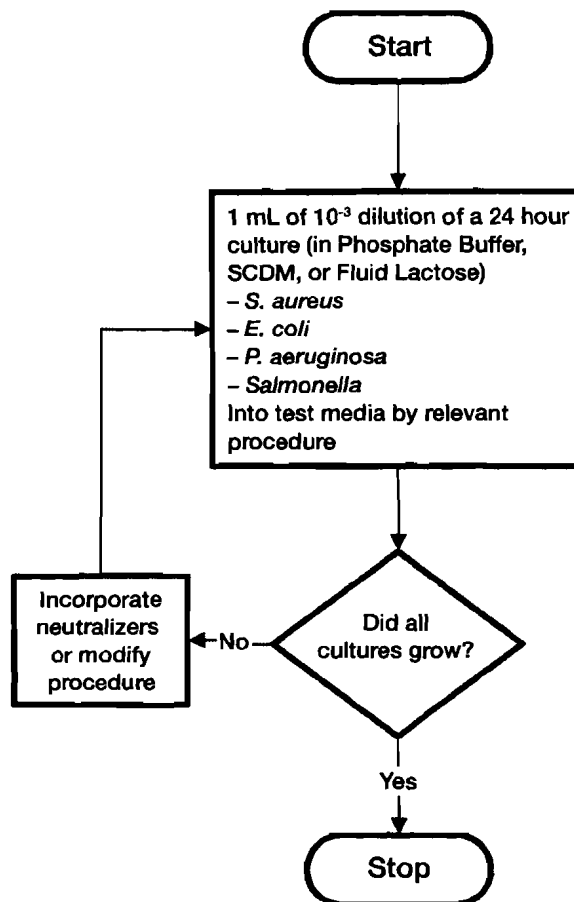
CAN THESE PRODUCTS BE MATRIXED?

If it is determined that the method suitability tests must be repeated, then the next question is one of logistics. Some companies have reported hundreds of products affected by this harmonization and the resultant changes in the test methods. Many products can be thought of as existing in families, with minor variations in formulation or unit fill volume. One approach to the issue of having to perform method suitability testing on so many products is to try to put together a testing plan that considers these product family groups. All products may eventually have to be re-validated; this depends on the product line and the company (and the US Food and Drug Administration) philosophy. However, this is one way to at least begin to organize the task.

IS THIS TEST REALLY NECESSARY?

As we look at the opportunities provided to us by the harmonized Microbial Limits Tests, a legitimate question has to be: Is this test doing any good? Is there a benefit to the patient or to the company in performing this test? I realize this sounds like heresy, but it is a legitimate question. As we discuss this topic, understand that the author is not a lawyer, nor does he speak for USP, FDA, or any other regulatory organization with an acronym. The advantage to having written regulations is that anyone can read them. The following discussion is presented only for consideration and is not meant as a specific recommendation for any course of action.

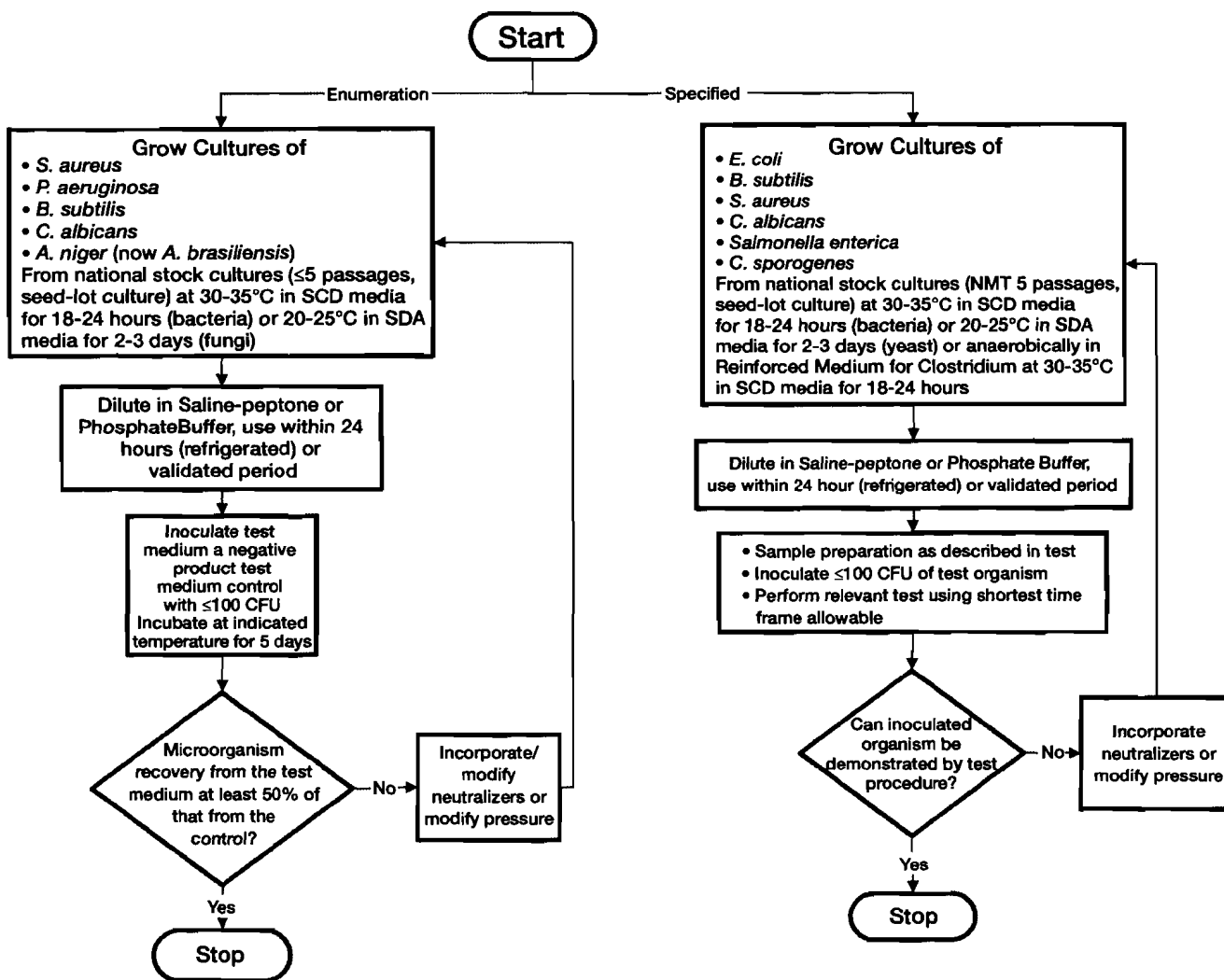
Figure 1: Overview of previous "preparatory testing" procedure for USP microbial limits tests.



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, <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 that states the following:

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Figure 2: Method suitability flowcharts for harmonized method suitability testing.



“... 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 the GMP? One aspect is the recognition of USP by CGMP, which states in 21 CFR 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” (9).

So the perception is that if you follow the USP you are following GMP. While this is 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 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 (10). However, this position is not supported by FDA (11) nor by USP 1982 (12) and obviously hasn’t been for decades. In fact, addition characterization of isolates from non-sterile products and their analysis is now an explicit expectation of the harmonized chapters. This direct instruction can be found in the harmonized informational chapter <1111> (8), which states:

“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 technol-

ogy 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 (13).

Bringing this academic discussion into the real world, FDA has a solid track record of enforcing this requirement. A review of recalls from 1998 through 2006 (14) 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.

We have to consider the original question—Is the microbial limits test really necessary? 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 a monograph requirement. Whatever the decision as to the need for the Microbial Limits Tests as a finished product release test, it is not sufficient to ensure compliance with the expectations of either 21 CFR 211 or USP <1111>.

WHERE DOES THIS LEAVE US?

I would urge the re-validation of existing tests to current standards and level of detail. It is extremely unlikely that method suitability studies performed years ago will be documented in sufficient detail to show compliance with the expectations written into the harmonized procedures even if the tests were performed identically. The first step in this process would be to perform an analysis of currently marketed products for relationships among them. A plan can then be developed from this product analysis to perform method suitability studies as efficiently as possible.

Whatever the situation with the re-evaluation of the suitability of the test method, it must also be clearly held in mind that this is not the microbial quality expectation for marketed, non-sterile product in the United States as described by 21 CFR 211, FDA regulatory precedent, and the internationally harmonized USP <1111>. The manufacturer is expected to know what microorganisms are present in their non-sterile medications and have confidence that those microorganisms will not imperil either the patient using the medication nor the quality of the medication on long-term storage.

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ARTICLE ACRONYM LISTING

CGMP	Current Good Manufacturing Practice
EP	European Pharmacopeia
FDA	US Food and Drug Administration
JP	Japanese Pharmacopeia
NF	National Formulary
TAMC	Total Aerobic Microbial Count
TYMC	Total Yeast and Mold Count
USP	United States Pharmacopeia