

Developing an Information Chapter in the USP to Demonstrate Equivalency in Microbiological Methods

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The importance of microbiological control in the pharmaceutical and medical products industry, be it for sterile or non-sterile products, excipients, raw materials or drug substances, makes it necessary to have an in-depth understanding of its role in compendial requirements. Readers of monographs in USP 25⁽¹⁾ will frequently encounter compendial requirements pertaining to microbiology such as these from the monograph for Insulin Injection (page 913, USP 25):

“Bacterial Endotoxins <85>—It contains not more than 80 USP Endotoxin Units for each 100 USP Insulin Units.

Sterility <71>—It meets the requirements when tested as directed for Membrane Filtration Method under Test Procedures.”

Similar references may be found in various monographs in USP 25 pertaining to **Microbial Limit Tests <61>** as well.

In each case, the monograph specifies a specific general chapter within USP 25 with a number below 1000. Such chapters include general requirements for tests and assays, and describe how to test the compendial article for compliance with (as the case may be) requirements for bacterial endotoxins, microbial limits and sterility. General chapters are an efficient means of presenting methodologies for common requirements such as for sterility. For example it is not necessary to provide detailed instructions on how to perform the compendial sterility test in every monograph that has a sterility requirement. Instead, the monograph can refer to a general test chapter (<71> in the case of sterility testing). However, it is permissible for an individual monograph to provide test specifica-

tions beyond those given in the general test chapter. In such cases, the specific test information in the monograph supercedes that in the general test chapter.

It is important to understand what is meant by compliance with the compendial requirements for an official article in USP 25. The following text from General Notices in USP 25 is important in this respect:

“Every compendial article in commerce shall be so constituted that when examined in accordance with these assay and test procedures, it meets all of the requirements in the monograph defining it. However, it is not to be inferred that application of every analytical procedure in the monograph to samples from every production batch is necessarily a prerequisite for assuring compliance with Pharmacopeial standards before the batch is released for distribution.”

Important points to be gleaned from the preceding text are that a compendial article is expected to satisfy compendial requirements when tested according to the compendial methods, but not every batch of product must be so tested. In other words, the principal purpose of the methods is not to serve as batch release tests. What is essential to realize is that should a product, for which a monograph exists in USP, be tested, it must meet all the requirements contained in the monograph.

The essential role of test methodologies provided in general chapters with numbers below 1000 is to describe how to test compendial articles for compliance with the requirements specified in USP monographs. One question that often arises is: Does the compendial method as given in the general chapter have to be used to demonstrate compliance, or could an alternative method be employed instead? In the case of microbiological testing, there are numerous reasons why an

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alternative test method may be desired. These reasons may include less expense, less time required for results, less labor intensiveness, easier or simpler test procedures, improved accuracy, precision, automation, etc. With some biotechnology products, the time required to complete microbiological testing may exceed the shelf life of the product. Other products may be made in such small quantities because of their cost that the compendial methods may be prohibitive. Still other products, such as those made from autologous cells that may need to be administered as rapidly as possible to a patient, may not permit standard microbiological testing for a combination of the previous reasons. The following text from General Notices in USP 25 provides the compendial view of the answer for the question of alternative methods:

“Compliance may be determined also by the use of alternative methods, chosen for advantages in accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction or in other special circumstances. Such alternative or automated procedures or methods shall be validated. However, Pharmacopeial standards and procedures are interrelated; therefore, where a difference appears or in the event of dispute, only the result obtained by the procedure given in this Pharmacopeia is conclusive.”

It is clear from the preceding text that alternative methodologies are permissible to demonstrate compliance, given proper validation and comparison with the compendial test, but not where a dispute arises between the results from the compendial method versus the alternative method. Note that the FDA must use the USP test to show noncompliance. It is also evident that the compendial methods serve as the referee tests, should a divergence arise between results from the compendial test and an alternative method. Therefore, it is essential that an alternative method be extensively validated in order to minimize as far as possible the chance that such a divergence in test results could arise.

In USP 25, there are general information chapters (chapters numbered 1000 or higher) that provide guidance on the validation of compendial test methods. Chapter <1225> Validation of Compendial Methods is often referred to in the development of compendial methods. The following text from the chapter is informative:

“Recognizing the legal status of USP and NF standards, it is essential, therefore, that proposals for adoption of new or revised compendial analytical methods be supported by sufficient laboratory data to document their validity.”

Thus, this chapter provides guidance for the development of either new or revised compendial analytical methods, primarily chemical in nature. Of interest, and as noted in chapter <1225>, the chapter harmonizes as far as possible with the Tripartite International Conference on Harmonization (ICH) documents Validation of Analytical Procedures⁽²⁾ and the Methodology extension text⁽³⁾. The harmonization is not complete, due in part because of different uses of terminology.

Another chapter in USP 25 that provides guidance on validation, in this case, specific to microbiological testing, is <1227> Validation of Microbial Recovery from Pharmacopeial Articles. As indicated by the chapter’s title, for methods dependent upon microbial recovery, guidance is provided for validating that recovery has occurred to produce valid results. General test chapters <51>, <61>, and <71> all depend on adequate microbial recovery. In chapter <1227>, information is provided related to the neutralization of antimicrobial properties that may exist within a compendial article.

While chapter <1225> provides information on a wide array of aspects pertinent to the validation of compendial methods, it is most directly relevant to chemical tests. Although chapter <1227> directly pertains to microbiological methods, it does not broadly apply to the development of alternative tests. Again, its emphasis is on validating microbial recovery. Recognizing that extensive validation is essential for any method under consideration as an alternative to a compendial one, and given the lack of a chapter akin to <1225> that is broad in its consideration of validation requirements but specifically focused on microbiological methods, the Analytical Microbiology Committee of Experts has undertaken the development of a new general information chapter. This effort was aided by a recent publication from the PDA which provides focused guidance on validation of alternate microbiology tests entitled “Evaluation, Validation and Implementation of New Microbiological Testing Methods”⁽⁴⁾.

The proposed new chapter that grew out of this initiative, entitled Validation of Alternative Microbiological Methods, has been published as a Pharmacopeial Preview in Pharmacopeial Forum⁽⁵⁾. A word of explanation is in order about the public review process employed by the United States Pharmacopeia. The first appearance of a new chapter typically is as a Pharmacopeial Preview. The intent of this publication is to solicit input from interested parties. The responsible subcommittee (in this case the Analytical Microbiology Committee of Experts) then reviews the comments, modifies the draft, and submits the amended version as an In-Process Revision. There may be several versions of the proposed chapter published as In-Process Revisions until the responsible subcommittee is satisfied that a suitable chapter has been completed. That draft is then voted on by specified members of the Committee and/or Council of Experts, and if accepted, is included as an official chapter in the next revision. As you can see, the recent publication of this proposed chapter as a Pharmacopeial Preview denotes its early stage in the development process.

This proposed chapter is numbered <1223>, and therefore is considered informational and not mandatory in that it is not required by a monograph. The goal of the chapter is to fill in the gap in guidance on validation of compendial methods specific to microbiological tests. As stated in the chapter: “It is the purpose of this general information chapter to provide

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guidance on the demonstration of the suitability of alternative microbiological methods to be used as part of, or in lieu of, compendial assays.”

Unless and until a compendial microbiological requirement appears that is not addressed by the current general chapters <51>, <61> and <71>, any new microbiological method would have to be treated as an alternative method. Moreover, it would be essential to compare this alternative method to the existing compendial method. The goal is to ensure through extensive validation that the results obtained from the alternative method will lead to the same scientific conclusion as the results from the compendial method.

It is possible that the alternative method under consideration would not be intended to replace a compendial method totally, but rather serve as an alternative to a portion of the compendial method. For example, and as stated in <1223>, there are a variety of techniques whereby the presence of viable microorganisms may be detected. Such techniques may be applicable to a range of compendial methods in part. Chapter <1223> describes how one may follow the membrane filtration approach in <71> Sterility Tests to the point where potentially viable microorganisms are recovered on the filter, but then the presence of these microorganisms might be detected through some means other than growth in media. Among the alternatives to growth in media are impedance measurements, ATP bioluminescence, flow cytometry, head space gas measurement, and epifluorescent vital dyes. In a case where only a portion of the compendial method is considered for replacement by the alternative technique, only the portion of the compendial method to be substituted would require comparison to the overall compendial method. In this case, the comparison would be between recovery of viable cells from the filter in liquid medium (TSB and/or FTM) and evidence of viable cells as demonstrated by the alternate method.

The three principal types of microbiological determinations currently required for various compendial articles are the detection of any viable microorganisms, the quantification of the number of microorganisms present, and the identification of microorganisms. Chapter <1223> discusses each in turn. The sterility test is the most common example of a qualitative test. Quantitative microbiological tests in USP 25 employ standard plate counting where possible. If not, other methods such as the most probable number method are used. The current compendial methods employ primarily morphological and biochemical evaluation in identification tests. Given three basic applications for microbiological testing, chapter <1223> provides three separate sets of validation and comparison criteria.

Chapter <1223> provides a table listing of validation parameters (accuracy, precision, specificity, detection limit, quantification limit, linearity, range, robustness and ruggedness) and an indication of which of these parameters are applicable to the three basic types of compendial microbiological tests.

Following the table, the chapter discusses the definitions of these terms as they apply to each of the basic test types. Following the definition for each parameter, guidance is provided on how to determine the value for the parameter within the context of each basic test type.

For qualitative methods, “accuracy” relates to the closeness of the results from the alternative method to the compendial one. “Precision” is related to the degree of agreement between the compendial and alternative methods when the methods are applied repeatedly on different lots of the same product. As stated in <1223>: “The alternate method must provide at least as high a recovery as the compendial method.” “Specificity” for a qualitative test refers to its ability to detect a range of microorganisms that may be present in a compendial article. “Detection limit” refers to the lowest number of microorganisms that are detectable without concern for accurate quantification. The parameters “quantification limit”, “linearity” and “range” are not applicable to qualitative microbiological tests. “Ruggedness” is related to the precision of test results when the same samples are analyzed under a range of normal test conditions. The range of normal conditions could include different operators, instruments, laboratories, etc. “Robustness” is somewhat similar to ruggedness, but actual methodological parameters are varied in a small but deliberate fashion.

“Ruggedness” and “robustness” are highly significant from a compendial perspective. For a manufacturer, it is essential to use methods that are not so exquisitely sensitive to minor variations in normal test conditions, or to minor experimental parameter variations, that the reliability of the test results relative to the compendial results could come into question. Moreover, if the intention of the alternative method is hoped to be the eventual replacement of the compendial method with the alternative method, it should be remembered that the methods of the USP are used in many countries with many potential variations encompassed by ruggedness and robustness evaluation.

Next to be considered in <1223> are the validation parameters in the context of quantitative methods. Definitions for accuracy, precision, specificity are provided as before, now tailored to these types of tests. “Detection limit” is not applicable for quantitative tests, but “quantification limit” is applicable. It is the lowest number of microorganisms that may be detected with acceptable accuracy and precision. Likewise, “linearity” and “range” are applicable to quantitative tests. “Linearity” refers to the ability of a method to produce results that are proportional to the concentration of test microorganisms within a sample (within a given range). “Range” relates to the interval between the upper and lower number of microorganisms determined precisely, accurately, and with linearity. “Ruggedness” and “robustness” are defined relative to quantitative tests as well.

Chapter <1223> concludes with a discussion of the validation parameters relative to alternative methods for microbial identification. There are definitions provided for “accuracy” and “precision”. “Specificity”, “detection limit”, “quantitation limit”, “linearity” and “range” are not applicable to identification tests. Definitions for “ruggedness” and “robustness” relative to identification tests are also provided.

The definitions for the listed validation parameters, and methods for determining them, covered only superficially in this article, are covered in detail in chapter <1223>. It is essential, given the critical requirement for a thorough validation for any method conceived of as a partial or complete alternative for an existing compendial method, that no shortcuts be taken in their validation. The proposed chapter <1223> is intended to provide guidance on how to perform a validation of an alternative microbiological method in order to demonstrate that it is suitable for determining that a compendial article meets its compendial requirements.

Chapter <1223>, as was indicated earlier, is in the Pharmacopeial Preview stage. The next step will be for it to be published as an In-Process Revision in PF. It is essential that any party interested in this chapter provide comments as they see fit. Such comments are essential contributions to the process of continuous revision of USP that is designed to result in monographs/general chapters/etc. that more strongly meet the needs of individuals with interest in the compendia. ■

References

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