Harmonized Microbial Limits Methods

How will new methods impact the industry?

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Prior to 2006, the United States Pharmacopeia (USP), European Pharmacopoeia (EP), and Japanese Pharmacopoeia (JP) used test methods to ensure microbial safety of non-sterile pharmaceutical products that were similar in intent, but widely variable in execution and acceptance criteria. These three pharmacopeia collaborated over the course of years to harmonize their testing methods and specifications for non-sterile pharmaceutical products. The pharmacopeias released two chapters in 2006 entitled "Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests" and "Microbiological Examination of Non-Sterile Products: Tests for Specified Microorganisms." These tests were historically referred to as "Microbial Limit Tests" and appeared in USP chapter <61> and JP 4.05. With the advent of the harmonization, the structure of the USP and JP were altered and the two chapters are now USP <61> and <62>, and JP 4.05 sections I and II. The EP had a structure similar to the harmonized chapters, and those tests are still found in EP 2.6.12 and 2.6.13.

The pharmacopeias set the scheduled implementation of the new harmonized methods to August 1, 2007, however the implementation was delayed to allow more time for users to comply with the new requirements. The new harmonized methods become official in 2009, but the official effective date varies depending on the pharmacopeia. Use of the new methods was required beginning January 1, 2009 for those products intended to meet EP requirements, beginning April 1, 2009, for the JP requirements, and beginning May 1, 2009 for the USP requirements. The harmonized methods are currently approved for optional use and may be implemented prior to these mandatory effective dates.

Each pharmacopeia previously described methods to demonstrate that antimicrobial activity of the test article is adequately neutralized and would not interfere with the validity of the test. The guidance was rather general, but this demonstration was known as "Preparatory Testing," recovery method validation, or something similar. The harmonized methods offer quite specific guidance for ensuring that the test article does not interfere with the testing. The new Microbiological Examination of Non-Sterile Products test methods contain significant changes over earlier versions, including new techniques, media, incubation times, and incubation temperatures. The harmonized methods also include three tests that are new to the USP and JP (tests for Bile-tolerant Gram Negative Bacteria, *Clostridia*, and *Candida albicans*), one of which is also new to the EP (test for *C. albicans*). Because of these alterations, it is necessary to re-evaluate the antimicrobial activity of products previously validated under the earlier methods.

To remain compliant with the pharmacopeial requirements, adequate neutralization of the antimicrobial activity of the products must be demonstrated using the harmonized methods and following the guidance in the harmonized chapters. The validation offers evidence that the conditions of the recovery method are appropriate for the specific product and that the sample preparation is not inhibitory to the representative organisms used in the test. Although there is an initial cost to

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perform a new recovery method validation on previously validated existing formulations, the long-term benefits are clear. Historically, to release a batch of product for use in Europe, the U.S., and Japan, replicate testing was required, doubling (or sometimes tripling) the cost to demonstrate microbial safety for the applicable markets. Also, acceptance criteria varied among the pharmacopeias. One ramification of varying requirements was a product that may have been acceptable for release in one market, but unacceptable in another. The USP <1111>, EP 5.1.4, and JP 12 have also harmonized the acceptance criteria for various non-sterile dosage forms (see Table 1). After harmonization, a batch of product that meets the acceptance criteria of these methods will now meet the requirements of any agency that recognizes the USP, EP and/or JP. ■

References

European Pharmocopoeia. "Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests." EP 6.2, chapter 2.6.12, 2008.

European Pharmocopoeia. "Microbiological Examination of Nonsterile Products: Tests for specified Microorganisms." EP 6.2, chapter 2.6.13, 2008.

European Pharmocopoeia. "Microbiological Quality of Non-Sterile Pharmaceutical Preparations and Substances for Pharmaceutical Use." EP 6.2, chapter 5.1.4, 2008.

Supplement I to the Japanese Pharmocopoeia Fifteenth Edition. "Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests." chapter 4.05, I, 2008.

Supplement I to the Japanese Pharmocopoeia Fifteenth Edition. "Microbiological Examination of Non-Sterile Products: Tests for specified Microorganisms." chapter 4.05, II, 2008.

Supplement I to the Japanese Pharmocopoeia Fifteenth Edition. "Microbial Attributes of Non-sterile Pharmaceutical Product." chapter 12, 2008.

United States Pharmacopeia. "Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests." USP 31, chapter <61>, 2008.

United States Pharmacopeia. "Microbiological Examination of Nonsterile Products: Tests for specified Microorganisms." USP 31, chapter <62>, 2008.

United States Pharmacopeia. "Microbial Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Use." USP 31, chapter <1111>, 2008.

Table 1: Test specifications by product type

Dosage Form	Total Aerobic Microbial Count	Total Combined Yeasts/Mold Count (CFU/g or mL)	Absence of Specified Microorganisms *
Non-aqueous preparations for oral use	10^3 CFU/g or mL	10^2 CFU/g or mL	*Escherichia coli; Salmonella sp.* (products containing unprocessed animal, plate or mineral ingredients)
Aqueous preparations for oral use	10^2 CFU/g or mL	10^1 CFU/g or mL	*Escherichia coli*
Rectal products	10^3 CFU/g or mL	10^2 CFU/g or mL	------
Oromucosal, Gingival, Cutaneous, Nasal, and Auricular use	10^2 CFU/g or mL	10^1 CFU/g or mL	*Staphylococcus aureus; Pseudomonas aeruginosa*
Vaginal use	10^2 CFU/g or mL	10^1 CFU/g or mL	*Staphylococcus aureus; Pseudomonas aeruginosa; Candida albicans*
Transdermal patches	10^2 CFU/patch	10^1 CFU/patch	*Staphylococcus aureus; Pseudomonas aeruginosa*
Inhalation use	10^2 CFU/g or mL	10^1 CFU/g or mL	*Staphylococcus aureus; Pseudomonas aeruginosa;* Bile-tolerant Gram-negative bacteria
Substances for pharmaceutical use (excipients)	10^3 CFU/g or mL	10^2 CFU/g or mL	------

*It may also be necessary to test for other organisms depending on the nature of the starting materials and manufacturing process.