Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Review Article

Received on: 08-07-2016
Accepted on: 12-07-2016
Published on: 15-08-2016

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Harmonization in Microbial Limit Test of USP and EP

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ABSTRACT

The harmonized versions have been published and it's continuing till both the country is ready for harmonization. Japan is also included in harmonization process. The harmonized chapters do not differ in most case from the drafts published in 2003 .This article briefly describes harmonized USP chapters {61} microbial enumeration , {62} absence of specified micro organism, {1111} microbiological attributes to no sterile pharmaceutical product .The format of USP chapters changes drastically with this harmonization whereas the microbial limit tests were two chapters in USP 29(5,6) they are now modified now in modified version to copy the European version. It is most important to the people who want to carry out the tests in both the country and want to export product in both country. The implementation of the tests was in different schedule in US and in Europe.

Key-words: United States pharmacopeia USP, European pharmacopeia EP; FDA; Harmonized microbial limit test; micro organism; Japanese pharmacopeia.

Cite this article as:
Dilip Maheshwari, Pragati Vanavi, Harmonization in Microbial Limit Test of USP and EP, Asian Journal of Pharmaceutical Technology & Innovation, 04 (19); 2016, 61-70 . www.asianpharmtech.com

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Introduction

The Harmonization in microbial limit tests are used to evaluate raw material and non sterile product for acceptable microbial quality. Microorganisms can also affect the health of patient, by knowing the amount and type of microorganisms present in product is important to patient health and safety. Implementation of this harmonized test has been done in United States from 2007 while in case Europe implementation was scheduled depending upon situation:

1. Substances included by a monograph specification: use the methods of the *European Pharmacopoeia* (A) until the monograph is revised and implemented.
2. Substances not included by a monograph specification: use either the methods of the *European Pharmacopoeia* (A) or the harmonized methods (B) until January 2010. From January 2010: use harmonized methods (B)
3. Preparations: use either method of the European Pharmacopoeia (A) or the harmonized method (B). For new preparations, use of harmonized method

Stages of pharmacopoeial discussion group process

1. Identification of item to be harmonized
2. Investigation into existing text.
3. Proposal for the expert committee review.
4. Official inquiry.
5. Consensus
   5. A. Provisional
   5 B. Draft sign off
6. Regional adoption and implementation.
   6 A. Adoptions
   6 B. implementation.
7. Inter-regional implementation.

Harmonized chapter number and details. Table 1.1

US pharmacopeia	European pharmacopeia
(61) Micro biological examination of non sterile product; microbial enumeration test.	2.6.12. Micro biological examination of non sterile product; microbial enumeration test.
(62) Micro biological examination of non sterile product; tests for specified micro organism.	2.6.13. Micro biological examination of non sterile product; test for specified micro organisms.
(1111)Micro biological quality of non sterile pharmaceutical product.	5.4.1 Micro biological quality of non sterile pharmaceutical product.

Chapters in brief.

USP ‹61› "Microbial Enumeration"

The microbial enumeration test is a basic, simple design to count the number of colony-forming units (CFUs) in a non sterile product or raw material. The preferred method is to put the material into a solution and then plate, the aliquots to determine the CFUs/g (or mL) of initial material. If the product cannot be put into a solution, the most probable number (MPN) method has several requirements to use. A full explanation of the MPN method is outside the scope of this article, but interested readers can refer to the discussion in the US Food and Drug Administration's *Bacterial Analytical Manual* (8).

The technique of plating can be pour plate, spread plate, or material filtration and then placing the membrane filter on an agar plate surface. The membrane filtration method should only be used when few CFUs are predictable to be found in the material to be tested. Though membrane filtration is a good technique to test a large volume of liquid, it can only count as many as 100 CFUs/membrane.

The harmonized method provides much more feature than any of the current pharmacopoeial methods in terms of representative method suitability (method validation) and media-growth endorsement.
USP ‹62› "Absence of Specified Microorganisms"

There is a major argument in the United States over the intention of this evaluation. FDA is bound by the concern articulated in the Code of Federal Regulations (21 CFR 211.113 and 21 CFR 211.165) relating to the importance of "objectionable microorganisms." This issue is addressed in the final section of this review because the harmonized Chapter ‹1111› deals with "other organisms."

Tables 1.2 present the offered "Microbial Limits—Absence of Specified Microorganisms" tests from the current USP and Pham Euro, as well as the coordinated document. It is offered as an aid to evaluation, and may assist in determining whether revalidation of method appropriate studies is needed. It should be noted that this harmonized chapter represents a true compromise by all parties, with major changes from the current USP, Pharm Eur, and JP chapters. Table 1.4 provide guidance about the media-growth promotion prospect of the new chapters.

USP ‹1111› "Microbial Quality": a new compendial consideration of "other organisms"
Chapter ‹1111› "Microbial assessment of Non sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use" is a relatively short section that has a major impact. For the US reader, the allowance for twice the specification in observed results is noteworthy. But, this is not the foremost change.
Before the introduction of the harmonized Chapter ‹1111›, USP was only interested in specified organisms. These organisms are specified in monographs. But, FDA has been concerned about offensive organisms. The "Control of Microbiological Contamination (a)" section of 21 CFR 211.113 states, "Appropriate written procedures, designed to prevent offensive microorganisms on drug products not required to be sterile, shall be recognized and followed." This is shatterproof by 21 CFR 211.165 which states in the section "Testing and release for distribution... (b) There shall be proper laboratory testing, as necessary, of each batch of drug product required to be free of offensive microorganisms."
Thus, industry has had a setback. The USP monograph for a product (as provided in the current National Formulary [NF]) may require the "Absence of Pseudomonas aeruginosa." A test in the "Microbial Limits" chapter demonstrate the absence of P. aeruginosa. Although this test may be needed to demonstrate conformity with the monograph requirements laid out in the National Formulary, it does not assemble FDA's concern that all microorganisms in a non sterile product should be acceptable to the product and the target population (i.e., are not "offensive").

DATA AND REVIEW
The demonstration of method suitability should be performed using the test organisms listed (see Tables) in harmony with the recommendations found in *USP* Chapter ‹1227› (9). Growth promotion is an area of some indistinctness in the compendial text. Although media-growth promotion is not described in the tests, it is required. *USP* Chapter ‹1117› (10) provides aid in designing the studies using 10–100 CFUs of the challenge organisms.

A major concern of many quality control workers is whether the changes in the harmonized chapter will require the revalidation of existing assays to meet harmonized test requirements. Several considerations might lead to revalidation: a required change in media, in the volume of material required for testing, or in general testing conditions. It is difficult to settle on whether all product types would require revalidation, and thus a summary table (see Table) is included in this article to describe the critical
Aspects of the current "Microbial Limits Tests (Enumeration)" and the draft harmonization text. This table is provided only as an aid. The decision of whether or not revalidation is necessary rests with each individual flair for its particular products.

Material and method:
Here are tabulated form of harmonized method and criteria for tolerant limits. Various chapters that are harmonized between USP and EP, their methods, accepted microorganism and absence of microorganism(table 1.4). Various nutritive, selective and indicative media (table 1.5)

Table 1.2 Aspects of the current and harmonized microbial limit test (enumeration).

Issue	US Pharmacopeia	European Pharmacopeia	Harmonized
Chapters	{61}	2.6.12	
Additional Control	None	• Use sterile sodium chloride and peptone solution ph 7.0 as a test preparation to test : • Sterility of medium • Sterility of diluents • Aseptic performance of the test.	Use sterile diluents as a test preparation for each batch of diluent to verify testing condition.
Interpretations Result	• Must meet Specification • Retest allowed using 25g Sample.	Must be within five fold of specification for product.	Must be within two fold of specification for product.

TABLE 1.3 Aspects of the current and harmonized microbial limits tests (enumeration)

Issue	US pharmacopeia 61	European pharmacopeia 2.6.12	Harmonized
MGP, organism for t.soy	s. aureus e.coli p.aeruginosa	s.aureus(ATCC) E.coli(ATCC) B.subtilis(ATCC)	S.aurues(ATCC) P.aeruginosa(ATCC) B.subtilis(ATCC)
MGP, organism for s.dextrose	NOT mentioned	C.albicans (ATCC) A.niger(ATCC)	C.albicans (ATCC) A.niger(ATCC)
MGP methodology	Not detailed	Use <100CFU/Media	Use <100CFU/Media Count must be within 50% control.
Media sterility check	Not detailed	Combined with negative product control.	Recommended.
Suitability of counting method	Inoculate diluted Stop efforts after particular if micro organism cannot recovered	Must show recovery in presence of product.	Use <100CFU/Media Instruction for neutralization of antimicrobial activity Count must be within 50% control Stop efforts after particular if micro organism cannot recovered

Sampling plan	Not detailed	Batch size Health hazards Product characteristics Expected level of contamination.	Not detailed Volume depands on Active agent in low concentration Bulk materials Small batches
Sample volume	10 gms	10 gms	10 gms
Categories	Water soluble Water immiscible fluids,ointment,creams,waxes Fluids in aerosol form.	Water soluble Nonfatty product insoluble in water Fatty products Transdermal patches.	Water soluble Nonfatty product insoluble in water Fatty products Transdermal patches. Fluids in aerosol form
Methodology , membrane filtration	Not listed	Transfer to 1 gm to each of 2 filters(0.45 micrometer pore size) 3 100ml wash 1 filter on TAS INCUBATE AT 30-35^{0}C,1 on SDA Incubate at 20-25^{o}c for NMT 5 days Count plates with NMT 100CFU	Transfer validated amount of 2 filters Wash each filter with validated method TAMC filter is placed TSB at 30-35^{o}c for 3-5 days TYMC filter is placed on SDA at 20-25^{o}c for 5-7 days.
Methodology, pour plate	1 ml sample+15-20 ml liquefied agar Plate in duplicate Incubate TSA at 30-35^{o}c for 18-72hrs for TAMC incubate SDA AT 20-25^{o}c for 5 days fot TCY And mould count.	Add 1ml sample+15-20ml liquefied agar Plate in duplicate Incubate as above Count plate with no more than 300 CFU.	1 ml sample+15-20 ml liquefied agar Plate in duplicate Incubate TSA at 30-35^{o}c for 18-72hrs for TAMC incubate SDA AT 20-25^{o}c for 5 days fot TCY And mould count. Count from plates with<250 for TAMC and <50 cfu FOR TYMC.
Methodology, plate count; spread plate	Not listed	Add 0.1 ml sample in petri plate containing agar Plate in duplicate Incubate as above Count plate with not more than 300 CFU	Plate 0.1 ml in at least duplicate on TSA and SDA by spreading on surface of prepared plates. Incubate as above Count from plates <250 for TAMC and <50 for CFY for TYMC.
Methodology MPN	Assemble 14 tubes(9ml each) Perform 10 fold dilution into 12 tubes Incubate 14 tubes Negative control must be clear Read result from table	Use only for bacteria 10 fold dilution in 3 TT Inoculate 3 aliquotes of each dilution 1 g or 1 ml sample Incubate at 30-35 ^{o}c for 5 days Read result from table provided.	Plate 0.1 ml in at least duplicate on TSA and SDA by spreading on surface of prepared plates. Incubate as above Count from plates <250 for TAMC and <50 for CFY for TYMC.

Abbreviation:	MGP is media growth promotion, MPN is most probable number, SDA is sabouraud dextrose agar, TAMC is total aerobic microbial count, TSB is tryptic soy broth and TYMC is total yeast and mould count.

TABLE 1.4 Absence of specified microorganisms.

Issue	US pharmacopeia {61} current	European pharmacopeia2.6.13 Current	Harmonized
MGP organisms for T.soy	S.aureus E.coli P.aeruginosa Salmonella	S.aureus(ATCC) E.coli(ATCC) S.typhimurium. P.aeruginosa.	S.aureus(ATCC) P.aeruginosa(ATCC) E.coli(ATCC) Salmonella enteric spp Candida albican(ATCC)
MGP methodology	Not detailed	Use less than 100 CFU/media in mixture.	See table no 1.5 Use <100 CFU Colonies are compared for appearance and indication reaction.
Method suitability	-	Use less than 100 CFU/media in mixture All test must work in the presence and absence of the product.	Detailed instruction in table no 1.5
Media sterility check	Not detailed	Combined with negative product control.	Recommended
Test for S.aureus	Specimen up to 100 ml +TSB-incubate If growth,streak on vogel Johnson agar medium Compare colonies- if absent meets specification If present-must be coagulase test negative to meet specification	Specimen upto 100ml+TSB-incubate at 35-37^{0}C for 18-48 hrs. Streak on baird parker agarand incubate at 35-37^{0}C 18-72 hrs Examine for black colonies of garam positive cocci Confirm by biochemical test Coagulase Deoxyribonuclease test.	**Sample preparation** 1gm sample + TSB-incubate at 30-35^{0}C for 18-24 hrs **Selection and subculture** if growth-streak on mannitol-salt agar –incubate at 30-35^{0}C for 18-72 hrs examine colonies for morphology confirm identity of suspect colonies.
Test for P.aeruginosa	Specimen 100ml+TSB-incubate If growth streak on cetrimide agar medium Compare colonies- if absent meets specification If present-streak colonies onto P.agar medium for the detection of pyocyanin Compare colonies for additional agar medium If absent-meets specification, Confirm suspect colonies with oxidase test.must be oxidase to meet specification.	Specimen upto 100ml+TSB-incubate at 35-37^{0}C for 18-48 hrs. Streak cetrimide agar and incubate at 35-37^{0}C 18-72 hrs Pass –if no growth If growth-gram stain.if h\gram negative rods seen, transfer colonies to TSB and incubate at 40-43^{0}C for 18-24 hrs. if no growth product passes.	Sample preparation 1 gm sample+TSB-incubate at 30-35^{0}C for 18-72 hrs Selection and subculture if growth-streak on cetrimide agar –incubate at 30-35^{0}C for 18-72 hrs examine colonies for morphology confirm identity of suspect colonies

Test for Salmonelaa spp.	Specimen100ml+fluid lactose medium- incubate If growth-pipette 1 ml into 10 ml Fluid selenite medium Fluid tetrathionate medium Incubate 12-14 hrs Streak growth on both medium onto: Brilliant green agar Xylose-lysine-deoxycholate agar Bismuth-sulfite agar Incubate and examine for growth If present –examine for gram negative rods Stab-streak colonies with gram negative rods into a butt-slant of triple sugar-iron-agar: incubate the slants and examine for red slants and yellow butts, if seen product fails.	Subculter on atleast two agars Deoxycholate citrate agar Xylose-lysine-deoxycholate agar Brilliant grren-phenol red Examine colonies Stab-streak colonies with gram negative rods into butt slant of triple sugar-iron –agar eaxamine for red slants and yellow butts Confirm by serological and biochemical test.	
Test for E.coli	100 ml specimen+lactose medium-incubate If growth-streak onto macconkey agar medium-incubate and examine If present-transfer suspect colonies to methylene blue agar medium to Levine eosine-methylene blue agar medium and streak for single colonies. Incubate and examine-if growth-product fails	100ml specimen+TSB incubate 35-37^{o}C for 18-48 hrs Transfer 1 ml to 100ml macconkey broth and incubate 35-37^{o}C for 18-72 hrs Streak onto macconkey agar incubate 35-37^{o}C for 18-48 hrs If colonies seen confirm with suitable test.	100ml specimen+TSB incubate 35-37^{o}C for 18-24 hrs If growth Transfer 1 ml to 100ml macconkey broth and incubate 42-44^{o}C for 24-48 hrs If growth Streak onto macconkey agar incubate 35-37^{o}C for 18-72 hrs If colonies seen confirm with suitable test. If no growth –product pass
Test for bile tolerant gram negative baceria	None	None	Sample preparation 1 gm sample+TSB- incubate at 20-25^{0}C for 2-5 hrs Test for absence Suff. Volume to inoculate a suitable amount of mossel enterobacteriaceae enrichment broth- incubate at 30-35^{0}C for 24-48 hrs If growth-streak on violet red bile glucose agar medium –incubate 30-35^{0}C for 18-24 hrs Product pass-ifno red colonies Quantitative test Use sample prepared under

			'sample preparation' with dilution of 10-1,10-2,10-3 into mossel enterobacteriaceae enrichment broth incubate at 30-35^{0}C FOR 24-48 HRS Plate samples on violet red blue glucose agar medium. Incubate for growth.

TABLE 1.5 Nutritive, selective and indicative properties of media

Medium	Property	Test strains
Test for bile tolerant gram negative bacteria • Mossel enterobacteriacea enrichment broth • Violet red bile glucose agar medium	• Nutritive selective • Nutritive indicative	• E.*coli,* P.*aeruginosa,* S.*aureus* • E.*coli,* P.*aeruginosa*
Test for E.*coli* • Macconkey medium • Macconkey agar medium	• Nutritive selective • Nutritive indicative	• E.*coli,* S.*aureus* • E.*coli*
Test for salmonella • Rappaport vassilladis salmonelia enrichment broth • Xylose-lysine-deoxycholate agar	• Nutritive • Nutritive indicative	• Salmonella enteric spp, E.*coli* • Salmonella enteric spp, E.*coli*
Test for P.*aeruginosa* • Cetrimide agar medium	• Nutritive selective	• *P.aeruginosa, E.coli*
Test for S.*aureus* • Mannitol salt agar medium	• Nutritive selective	• S.*aureus, E.coli*
Test for clostridia • Reinforced medium for clostridia • Coloumbia agar medium	• Nutritive • Nutritive	• C.*sporogenes* • *C.sporogenes*
Test for candida a*lbican* • Sabouraud-dextrose medium • Sabouraud –dextrose agar medium	• Nutritive • Nutritive indicative	• C.*albican* • *C.albican*

The FDA concern

If a company's product sanction to market submission states it will test the finished product by the "Microbial Limits Tests," FDA will enforce the good manufacturing practices (GMPs) condition that it must do so. This is purely a GMP concern. Nonetheless, the agency has been absolutely clear about its concern over offensive microorganisms in the product and that testing to the *USP* chapter might be necessary, but it is not sufficient to demonstrate satisfactory microbial quality. In fact, in the 1993 instructional guide for inspections of quality-control microbiology laboratories (11), FDA points out several issues that has occurred by particular contaminants of non sterile medicines that compromised patient health. The document notes that the *USP* provides methods for specific organisms, but not all offensive organisms and FDA strongly recommends all organisms are identified to settle on which are acceptable and which are offensive. This section of the guidance concludes:

The importance of identifying all isolates from either or both Total Plate Count testing and enrichment testing will depend upon the product and its intended use. Obviously, if an oral solid dosage form such as a tablet is tested, it may be acceptable to identify isolates when testing shows high levels. However, for other products such as topical, inhalants or nasal solutions where there is a major concern for microbiological contamination, isolates from plate counts, as well as enrichment testing, should be identified.

Concerns for microbial contamination, to understand, we must consider the history on this matter. As early as 1942, *USP* had a test for the "Bacteriological Examination of Gelatin" . But, most non sterile medications in the United States were not required to assay for microbiological quality attributes until the introduction of the "Microbial Limits Tests" in 1970. In the late 1960s, several outbreaks of disease were traced back to pathogen-contaminated medications, which encouraged increased awareness to the microbial content of non sterile drugs. Later in the 1980s, a series of articles described contamination by *P. cepacia* (currently *Burkholderia cepacia*) and its survival in disinfectants. This concern led to the addition of requirements in the 21 *CFR* to ensure that no objectionable organisms are in product released to market.

The compendial concern

As early as 1982, the *USP* is on record for verifying that the demonstration of "absence of objectionable microorganisms" is not the intent of the chapter. In a one-page Stimuli to the Revision Process , the microbiology committee of the time states:

The tests described in the Microbial Limits Tests ‹61› were not designed to be all-inclusive, i.e., to detect all potential pathogens. To achieve this, an extensive text on laboratory uncovering of microorganisms would be required. The procedures in USP were designed to perceive the presence of specific "index" or "indicator" organisms. Nevertheless, the present chapter does not preclude the detection of *Ps. Cepacia* - the organism requires subsequent differentiation. The chapter does not provide specific methods for this, nor does it provide procedures for detecting thousands of other potentially pathogenic organisms. Individual monographs include requirements for limits on total aerobic counts and/or absence of one or more of the four selected "indicator" organisms. The chapter on Microbial Limits Tests provides methods to assure that one may test for those microbial requirements in the individual monographs.

Against this background, we now examine the short harmonized Chapter ‹1111›, which consists of two tables and a few paragraphs. A significant passage in this chapter reads:

In addition to the microorganisms listed in Table I [Table I is entitled "Acceptance Criteria for Microbiological Quality of Non sterile Dosage Forms"], 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, and the debilitated.
Use of immune suppressive agents, corticosteroids.
The presence of disease, wounds, organ damage.

Where necessary, 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.

The harmonized chapter does not provide instruction beyond this level, but do not overlook this "new" recommendation. Merely showing the absence of specified organisms is not adequate to make obvious the microbial quality of a non sterile product.

Conclusions

The US Pharmacopeia and the US Food and Drug Administration are in conformity about the quality of the microbial quality of non sterile pharmaceuticals: the product must be in safe hands for use for patients.. The internationally harmonized chapters provide a strong scaffold for this assurance. It would be helpful for manufacturer and sponsor of clinical trial as they do not require performing costly test repeatedly and can save cost as well time.

The introduction of these three harmonized chapters is likely to entail some concerns regarding revalidation of offered methodologies. Companies should put plans in place immediately for this work and show consistent progress toward this goal.

The *National Formulary* monograph requirements for the absence of specific organisms are a minimal condition and should not be taken as evidence that the product is suitable for sale from a microbiological viewpoint. Harmonized Chapter ‹1111› recommends the determination of the risk associated with "other organisms," which is in conformity with the FDA expectation for absence of "objectionable" organisms. The manufacturer is accountable for the quality and safety of the product marketed, and it is FDA's clear expectation (as described in *CFR*) that this will include a determination of the microbial safety (*i.e.,* the "absence of objectionable microorganisms") from the product. These positions have been publicly stated for decades and should not come as a bolt from the blue. The harmonized microbial limits tests only deal with the "absence of specified microorganisms" and leave the determination of the "absence of objectionable microorganisms" in the proficient hands of each company's appropriately educated and well-trained microbiology group.

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