# HARMONIZED PHARMACEUTICAL MICROBIOLOGY

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For many years now, microbiologists in Europe, Japan, and the US have been separately assuring the purity of pharmaceutical products and raw materials with specific techniques accepted in their respective compendia. The results generated from these differing procedures are generally all valid, as there are many proven ways to detect, enumerate , and culture indicator species.

The quality assurance implication of these distinct regional preferences is however, a potential inefficiency for manufacturers in a global market place. Current "business as usual" practices may now have the same product tested and validated three different ways in three different places with three different methods. As the only truly global pharmaceutical contract testing laboratory, SGS is in the business of technical harmonization, and recognizes the value of operational synchronization for ourselves and our global customers. We have spent the last few years successfully developing and implementing harmonized quality assurance procedures in our 14 Life Science Services - Quality Control Testing laboratories in North America, Europe, and Asia, and we are well positioned to help you with this latest compendial change.

The new United States Pharmacopeia (USP), European Pharmacopeia (EP) and Japanese Pharmacopeia (JP) harmonized microbial enumeration and qualification method has been available for some time; but the changes must be implemented by May 2009. Although this date is a year out, conversations with several customers indicate that not much preparation for the change has been executed. Depending on the product line, there may be a need to purchase additional equipment, media, and micro-organisms. Most manufacturers will need to re-validate test methodology. Advanced planning will ensure no delay in production and release schedules. We offer this article to help you perform your own gap analysis.

## A BRIEF SUMMARY OF NOTABLE CHANGES

For the US Market, the most obvious change is a split from one General Test Chapter to two. USP has added <62> Objectional Organisms to <61> Enumeration. This change allows for a greater focus and elaboration on the difference between qualitative and quantitative analysis. Much greater detail is devoted to enumerating bioburden, that is describing the method for counting the potential microbial population, as well as the validation of the count in the pharmaceutical material.

What was once called "Preparatory Testing or colloquially, the Prep Test" is now more properly referred to as "Verification of Suitability of the Method", a term that is more harmonious with the "system suitability" jargon used in the chemistry laboratory. The original US Prep Test instruction was vague: the user was instructed to demonstrate test species growth in the presence of the product but no specific instruction was offered for inoculum count or type. The new version is quite specific about initial inocula levels, test species, and pass/fail values. Manufacturers must review their historical Prep Test data and be sure plate counts were properly validated to today's standards.

The European Pharmacopeia was more specific regarding growth promotion and validation. The test species were clearly defined for plate count, and the inocula level was approximately 100 cfu. The pass criteria was defined as differing by not more than a factor of five from the calculated value from the inoculum (20% recovery rate) – cfu in inoculated sample plates must now be >50% of the count of the control plates. Again, European manufacturer's should review previous validation packages to updated pass criteria were met.

The Japanese Pharmacopeia had two chapters for Microbial Limits Testing: Chapter 35 "Microbial Limits Test" and Chapter 36 "Microbial Limits Test for Crude Drugs". The harmonized method does not make this distinction. Inoculum counts for validation were required to be 50 – 200 cfu, and a pass value was >20% of inoculum in presence of test sample. This has now been changed to <100 cfu inoculum and 50% recovery.

USP <62> Objectional Organisms streamlines some tests while complicating others. For example, the old USP scheme would have a microbiologist work through 4 days of selective enrichments and platings, ending with 6 isolated streak cultures to evaluate. The new method ends in 1 streak after 3 days. The good news for the US scientist is the method is simpler and in the long run will save labor and cost. The bad news is it requires training, new media, and revalidation for every product tested for Salmonella. There are additional changes to methodology for all of the indicator species.

From the European perspective, Salmonella testing is similarly streamlined. Where as 2 plates were previously streaked from a selective tetrathionate broth, now there is only 1 plate streaked (XLD) from a Rappaport Vassiliadis Broth. Incubation temperature ranges are changed for all of the indicator species.

The original JP method for indicator organisms closely follows the original USP. The same changes would apply to Japan as do to the US.

## DETAILED ANALYSIS:

A more detailed summary of the changes in the Harmonized Method as viewed from each of the pharmacopiea is offered in Table 1. Media have been abbreviated, refer to Appendix for media acronym deciferation.

### TABLE 1: SUMMARY OF CHANGES

| HARMONIZED METHODOLOGY | USP 30 <61> | EP 2007 | JP XV |
|---|---|---|---|
| **AEROBIC PLATE COUNT: TSA** | | | |
| Validation with enumerated test species:<br>*B. subtilis*<br>*P. aeruginosa*<br>*S. aureus*<br>*C. albicans*<br>*A. niger* | All new | Pass criteria defined | Pass criteria and inocula level defined |
| **YEAST AND MOLD COUNT: SDA** | | | |
| Validation with enumerated test species:<br>*C. albicans*<br>*A. niger* | All new | Pass criteria defined | |
| **BILE-TOLERANT GRAM NEGATIVE BACTERIA (COLIFORMS)** | | | |
| 1 gm sample size | All new | Unchanged | All new |
| 1:10 enrichment in TSB for 2 - 5 hours (20 – 25°C) | | (35-37°C) Lactose broth | |
| 10 mL to Mossel Broth for 24 – 48 hours (30 – 35°C) | | (35-37°C) | |
| Streak to Violet Red Bile Green agar for 18 - 24 hours (30 – 35°C) | | (35-37°C) | |
| **ESCHERICHIA COLI** | | | |
| 1 gm sample size | All new | Unchanged | All new |
| 1:10 sample prep | | Unchanged | |
| 10 mL to TSB for 18 – 24 hours (30 – 35°C) | | (35-37°C) | |
| 1 mL to MAC Broth for 24-48 hours (42 – 44°C) | | (43-45°C) | |
| Sub to MAC Agar for 18 – 72 hours (30 – 35°C) | | (35-37°C) | |
| **SALMONELLA** | | | |
| 10 gm TSB for 18-24 hours (30 – 35°C) | All new | Unchanged | All new |
| 0.1 mL to Rappaport Vasiliadis 18-24 hours (30 – 35°C) | | 1 mL to Tet | |
| Streak to XLD for 18-48 hours (30 – 35°C) | | Streak to 2 agars | |
| **PSEUDOMONAS AERUGINOSA** | | | |
| 1 gm sample size | 10 gm | Unchanged | 10 gm |
| 1:10 sample prep | Unchanged | Unchanged | Unchanged |
| 10 mL enrichment in TSB for 18-24 hours (30 – 35°C) | New | (35-37°C) | New |
| Streak to CET, incubate for 18 – 72 hours (30 – 35°C) | Unchanged | (35-37°C) | Unchanged |
| **STAPHYLOCOCCUS. AUREUS** | | | |
| 1 gm sample size | 10 gm | Unchanged | 10 gm |
| 1:10 sample prep | Unchanged | Unchanged | Unchanged |
| 10 mL to TSB for 18-24 hours (30 – 35°C) | New | (35-37°C) | New |
| Streak to MSA and incubate for 18 – 72 hours (30 – 35°C) | Unchanged | (35-37°C) | Unchanged |

**TABLE 1: SUMMARY OF CHANGES CONT.**

| | HARMONIZED METHODOLOGY | USP 30 <61> | EP 2007 | JP XV |
|---|---|---|---|---|
| **CLOSTRIDIUM (UNDER ANAEROBIC CONDITIONS)** | | | | |
| | 2 gm sample size | All new | unchanged | All new |
| | 2 x 1:10 saline | | Unchanged | |
| | Heat shock one aliquot | | Unchanged | |
| | 10 mL reinforced Medium for Clostridium for 48 hours (30 – 35°C) | | (35-37°C) | |
| | Streak to Columbia Agar and incubate for 48 hours (30 – 35°C) | | (35-37°C) | |
| **CANDIDA ALBICANS** | | | | |
| | 1 gm sample size | All new | All new | All new |
| | 1:10 sample prep | | | |
| | 10 mL of sample prep to SDB for 3 – 5 days (20 – 25°C) | | | |
| | Streak to SDA and incubate for 24-48 hours (20 – 25°C) | | | |

## GAP ANALYSIS

Do you need to re-validate? Probably. Which tests exactly? Table 1 helps illustrate the changes between Pharmacopeia. Table 2 highlights necessary components in a validation package. The questions asked will help you perform a gap analysis and determine which products and tests might be in need of update:

**TABLE 2**

| **AEROBIC PLATE COUNT : SOYBEAN CASEIN DIGEST AGAR** | **YES/NO** |
|---|---|
| Do you have data demonstrating recovery in 3 days of ≤*100 S. aureus* in product comparable to 50% of control? | |
| Do you have data demonstrating recovery in 3 days of ≤*100 P. aeruginosa* in product comparable to 50% of control? | |
| Do you have data demonstrating recovery in 3 days of ≤*100 B. subtilis* in product comparable to 50% of control? | |
| Do you have data demonstrating recovery in 5 days of ≤*100 C. albicans* in product comparable to 50% of control? | |
| Do you have data demonstrating recovery in 5 days of ≤100 *A. niger* in product comparable to 50% of control? | |
| **YEAST AND MOLD COUNT: SABOURAUD DEXTROSE AGAR** | **YES/NO** |
| Do you have data demonstrating recovery in 5 days of ≤*100 C. albicans* in product comparable to 50% of control? | |
| Do you have data demonstrating recovery in 5 days of ≤100 *A. niger* in product comparable to 50% of control? | |
| **BILE-TOLERANT GRAM NEGATIVE BACTERIA (COLIFORMS)** | **YES/NO** |
| Do you have data demonstrating recovery of ≤100 cfu *E. coli* in product utilizing Mossel Broth and Violet Red Bile Green Agar? | |
| **ESCHERICHIA COLI** | **YES/NO** |
| Do you have data demonstrating recovery of ≤100 cfu *E. coli* in product utilizing Tryptic Soy Broth, Maconkey Broth and Maconkey Agar? | |
| **SALMONELLA** | **YES/NO** |
| Do you have data demonstrating recovery of ≤100 cfu *Salmonella* in product utilizing TSB, Rappaport Vasiliadis and XLD? | |
| **PSEUDOMONAS AERUGINOSA** | **YES/NO** |
| Do you have data demonstrating recovery of ≤100 cfu *P. aeuginosa* in product utilizing TSB and Cetrimide Agar? | |
| **S. AUREUS** | **YES/NO** |
| Do you have data demonstrating recovery of ≤100 cfu *S. aureus* in product utilizing TSB and Mannitol Salt Agar? | |
| **CLOSTRIDIUM** | **YES/NO** |
| Do you have data demonstrating recovery of ≤100 cfu *C. sporogenes* in product utilizing heat shock techniques, reinforce Clostrida Media, and Columbia Agar? | |
| **CANDIDA ALBICANS** | **YES/NO** |
| Do you have data demonstrating recovery of ≤100 cfu *C. albicans* in product utilizing Sabouraud Dextrose Broth and Agar? | |

## SHOPPING LIST

Need a shopping list? Depending on your gap analysis, you may need to make some capital and supply expenditures before you can get started. Table 3 lists items that might need to be purchased to comply with the new harmonized method. Actual needs will of course vary with the laboratory and the products tested. The list details items that are new to the harmonized method as compared to previous pharmacopeia.

**TABLE 3: SHOPPING LIST**

| | USP/EP/JP |
|---|---|
| 40-45°C Incubator | |
| Heat shocking water bath | |
| Anaerobic jars or incubators | |
| Mossel Broth | |
| VRBGA | |
| MacConkey Broth | |
| Rappaport Vasiliadis Broth | |
| Reinforced Medium for Clostridia | |
| Columbia Agar | |
| Isopropyl myristate (for insolubles) | |
| Gauze (for patches) | |
| *B. subtilis* ATCC 6633 | |
| *C. sporogenes* ATCC 11437 | |
| *C. albicans* ATCC 10231 | |
| *A. niger* ATCC 16404 | |
| *P. auerginosa* ATCC 9027 | |
| *S. aureus* ATCC 6538 | |
| *E. coli* ATCC 8739 | |
| *S. enterica* NCTC 6017 | |

## THE SGS ADVANTAGE

SGS Microbiology laboratories are ready with the proper methods, equipment, validations, and training to help you meet the new harmonized microbiology criteria. This time of change is a good time to consider outsourcing these analyses, allowing the manufacturer to focus on its core competencies. In addition, if a manufacturer is not happy with current contract laboratory, this is a good time to re-validate with a lab focused on regulatory quality and on time delivery

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