

Harmonization of Compendial Microbial Limits

Are you ready?

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THE YEAR IS 1942. THE PHARMACEUTICAL microbiologist puts down his newspaper. The article about the new wonder drug penicillin was encouraging but worrisome; so effective, yet only enough product to treat a few hundred soldiers. He went to the lab, opened USP XII, and streaked the gelatin enrichments to Eosin Methylene Blue Agar in a screen for *Escherichia coli*, much as any pharmaceutical microbiologist would do today.

Fast forward to 1970. The pharmaceutical microbiologist puts down her journal. The article about restriction enzymes was encouraging but worrisome; imagine actually being able to manipulate human DNA. She went to the lab, opened USP XVII, and streaked the Selenite Cysteine broth to selective agar for *Salmonella* species isolation, much as a pharmaceutical microbiologist would do today.

Biological advances have been staggering in the last several decades: life-saving cures, life-altering biotechnology. Meanwhile microbiological methods to analyze these drugs have been relatively constant. Now the U.S. pharmaceutical microbiology laboratory is about to experience a paradigm shift. The first substantive change to USP <61> Microbial Limits chapter in a generation will be published in the *Pharmacopeial Forum* in August 2006. Because the impetus is global harmonization, it's not just the U.S. laboratory that will have to adjust; concessions in methodology are being made by the European and Japanese Pharmacopeia as well. The original Stage 4 Official Inquiry was published five years ago. But now imple-

mentation is just around the corner, less than a year away. Is your laboratory ready? Although the actual publication is not available at time of print, we know from the USP that the new tests will be substantively similar to what was published in the *Pharmacopeial Forum* Vol. 29 No. 5 Sept.-Oct. 2003. Changes published in that draft reveal the consequential validation implications which will result.

Enumeration of Bioburden

The most obvious change is that USP <61> Microbial Limits has been divided into two chapters: USP <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests, and USP <62> Microbiological Examination of Nonsterile Products: Tests for Specified Organisms.

The most significant alteration in the harmonized USP <61> Enumeration Tests is replacement of the former referred 'Preparatory Testing' with 'verification of suitability of the method.' The old Preparatory Test lacked a clear mandate for verification of enumeration. The new method details both:

1. growth promotion of the media (the ability of the media without product to support low numbers of typical test species) and

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2. suitability of the counting method (the ability of the media and product to support low numbers of typical test species)

Table 1 below outlines some of the characteristics of the Growth Promotion and Suitability Tests.

Both the agar used in the analysis and the sample dilution that will be poured with the agar, are to be inoculated with <100 cfu of each appropriate test species.

Table 1: Growth Promotion and Suitability of Enumeration Agars

Agar	Test Species	Incubation
Soybean – Casein Digest Agar Media	<i>Staphylococcus aureus</i> ATCC 6538 <i>Pseudomonas aeruginosa</i> ATCC 9027 <i>Bacillus subtilis</i> ATCC 6633	30 - 35° C not more than three days
Potato Dextrose Agar	<i>Aspergillus niger</i> ATCC 16404	20 - 25° C not more than five days
Sabouraud Dextrose Agar	<i>Candida albicans</i> ATCC 10231 <i>Aspergillus niger</i> ATCC 16404	20 - 25° C not more than five days

Both the sample dilution which will be poured with the agar, and the agar alone, are to be inoculated with <100 cfu of each appropriate test species. The test scheme (dilution, neutralization, media selection, media production) will be suitable if the count of the test plates do not differ by more than 50% or 0.3 log of a previous batch of media in the case of growth promotion, or of an agar plate without sample in the case of suitability. Preparation of the test strains is detailed. Also unique to the new harmonized method is a prescription for a negative control to verify the absence of media contamination.

These changes have significant implications on validation. USP 29 <61> details neither growth promotion nor preparatory testing for microbial enumeration. On the other hand, USP 29 <1227> Validation of Microbial Recovery does offer a neutralization validation strategy for agar plate counts. However, as an informal chapter, some labs may not be following it. My laboratory has been performing suitability of plate counts for years, and comments I've heard from client auditors indicate many drug manufacturers have as well. However, those laboratories that bypass these counts (or tests or plate counts) will soon need to incorporate such validation in order to remain compliant.

Details on neutralizing agents, filtration, and MPN methods are also included in the new chapters. In addition, sections on how to handle insolubles, fatty products, aerosols and transdermal patches are also added. The topic of sample size is also expanded, allowing some concessions on the previous 10 gm sample for bulk actives and small batches.

Specified Organisms

USP <62> details a process for evaluation of specified test organisms. It is worth noting the emphasis on "specified" microorganisms, as opposed to the

cGMP regulations concerning the absence of "objectionable micro-organisms" in 21CFR211.113. Similar to the current USP <61>, USP <62> details a scheme for culturing classes of contaminants. The actual impact of any isolate must be understood in the context of the

product and the intended consumer.

USP <62> also details growth promotion and suitability. Whereas USP 29 Preparatory Test allows for inoculation of a 10⁻³ dilution of an overnight culture to sample enrichment, the new harmonized method requires inoculation of

sample enrichment with <100 cfu. This represents a >5 log difference in sensitivity. Labs that have suitability data based on such high inocula will want to retest.

Also notable is the addition of "nutritive" and "selective" properties to the verification process. In other words, the microbiology lab will now have to show that selective agar not only recovers bacteria for which it is selective (nutritive) but also prevents microorganisms to which it is inhibitory (selective). Inoculation criteria similar to USP <61> are detailed, although the strict pass criteria of 0.3 log or 50% are replaced with "colonies comparable in number and size." A negative control verifying the absence of contamination is also new.

Perhaps the most striking change to USP <62> is the enrichment scheme for *Salmonella* and *Escherichia coli*. Formerly, these "enteric" contaminants were enriched in Lactose Broth while the "topical" contaminants (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) were cultured in Soybean-Casein Digest Medium. This split always seemed strange to me, as the enterics grow just as well as the topicals in the SCD. The new method prescribes having all of these "Specified" microorganisms enriched in SCD. But the scheme changes significantly after enrichment (see Table 2).

The new *Salmonella* scheme will save labor in streaking and transferring. The new *E. coli* scheme may require capital investment in a new incubator, as the MacConkey broth is incubated at 42-44°C. Except for the sample size, the *Pseudomonas aeruginosa* and *Staphylococcus aureus* selection schemes remain relatively unchanged. There are new sections for Bile Tolerant Gram Negative Bacteria, Clostrida, and *Candida albicans*. Interestingly, the troublesome Retest section of USP 29<61> was dropped.

Are you ready?

Preparation is needed to meet these new enrichment and validation requirements. There is no harm starting the validation now, presuming Preparatory Test results already exist for USP 29. Overtime hours will be needed by the lab to meet all the validation requirements. To determine what your laboratory needs, a gap analy-

Table 2: Comparison of Changes in *E. coli* and *Salmonella* Tests

	USP 29	Harmonized
<i>E. coli</i>	<ol style="list-style-type: none"> 10 gm to 90 mL Lactose, incubate Streak to MacConkey Agar, incubate If growth with typical morphology, transfer to EMB, incubate 	<ol style="list-style-type: none"> 1 gm to 9 mL SCD, incubate 1 mL to 100 mL MacConkey Broth, incubate. Streak to MacConkey agar, incubate
<i>Salmonella</i>	<ol style="list-style-type: none"> 10 gm to 90 mL Lactose, incubate 1 mL to 9 mL Selenite Cysteine and Tetrathionate, incubate Streak to Brilliant Green Agar, Xylose Lysine Deoxycholate Agar, Bismuth Sulfite Agar, incubate Stab streak Triple Sugar Iron Agar 	<ol style="list-style-type: none"> 1 gm to 10 mL SCD, incubate. 1 mL to 10 mL Rappaport Vassiliadis Salmonella Enrichment Broth, incubate Streak to Xylose Lysine Deoxycholate Agar, incubate Identify presumptive colonies

sis is suggested. A basic checklist to get you started is provided in Table 3 below. These are items found in the Harmonized method but not seen in the current method. Alternatively, outsourcing the testing to a contract analytical laboratory is a viable option. Since these changes are a result of harmonization with EP methods, contract labs with global operations will have already screened products by USP and EP, giving them a head start. ■

USP 29 NF 24 <1111> Microbiological Attributes of Nonsterile Pharmaceutical Products, 4/1/2006.

USP 29 NF 24 <1227> Validation of Microbial Recovery, 4/1/2006.

21CFR211.113: Good Manufacturing Practices, Control of Microbial Contamination.

References

Cundell, Anthony "Review of the Media Selection and Incubation Conditions for the Compendial Sterility and Microbial Limits Tests" *USP Pharmacopeial Forum* Vol 28 No. 6 [Nov-Dec. 2002].

USP Pharmacopeial Forum Vol 29 No. 5 [Sept. – Oct. 2003], pp 1714 – 1735. <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests, <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms.

USP 29 NF 24 <61> Microbial Limits Test 4/1/2006.

Table 3: Checklist for Harmonized Testing

- | | |
|--|---|
| <input type="checkbox"/> 42 - 44°C incubator | <input type="checkbox"/> <i>Bacillus subtilis</i> ATCC 6633 |
| <input type="checkbox"/> Saboraud Dextrose Medium | <input type="checkbox"/> <i>Aspergillus niger</i> ATCC 16404 |
| <input type="checkbox"/> Muscle Enterobacteriaceae Enrichment Broth | <input type="checkbox"/> <i>Salmonella enterica</i> ATCC 14028 |
| <input type="checkbox"/> Violet Red Bile Glucose Agar Medium | <input type="checkbox"/> <i>Clostridium sporogenes</i> ATCC 11437 |
| <input type="checkbox"/> MacConkey Medium | <input type="checkbox"/> Data for growth promotion of all media batches |
| <input type="checkbox"/> Rappaport Vassiliadis Salmonella Enrichment Broth | <input type="checkbox"/> Data for suitability of enumeration schemes |
| <input type="checkbox"/> Reinforced Medium for Clostridia | <input type="checkbox"/> Data for negative controls |
| <input type="checkbox"/> Columbia Agar Medium | <input type="checkbox"/> Review of product specifications |

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