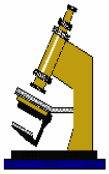
PMF
Pharmaceutical Microbiology Forum



PMF NEWSLETTER

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Inside this issue:

President's Message

"It is hard to believe that we are already in August! The PMF officers have been busy during the past few months not only dealing with new personal/career opportunities but also helping organize a microbiology conference for the Fall. Since Laura Valdes-Mora moved back to North Carolina, a new PMF address will be later posted on our web site. For now, you may continue to use the address in Panama City, Florida. I am glad to say that Laura will be living about 10 miles from my house!

A conference entitled "Microbiology in Support of Manufacturing, The Science Behind the Regulation" is scheduled for October 13-15, 2004. This meeting which will be held in Rochester, NY, is being presented by the Microbiology Network and the PMF. For more information and to review guidelines for abstract submission, go to http://highpeaks.us/abstracts.htm.

In this issue of our newsletter, we will be discussing the new proposed USP Chapter <1117> Microbiological Good Laboratory Practices. The USP is currently reviewing the many comments received on this document. In addition, its purpose and possible regulatory implications have been the focus of controversial discussions among pharmaceutical microbiologists.

Enjoy!

Lucia Clontz

Scott V.W. Sutton, Ph.D.

Dr. Sutton earned his B.S. in Genetics from the University of California at Davis, and his Masters and Ph.D. degrees in Microbiology from the University of Rochester (Rochester, New York). After a NIH postdoctoral fellowship at the Medical College of Virginia, he went to work for Bausch and Lomb until 1994.

In 1994, Dr. Sutton accepted a position at Alcon Laboratories (Fort Worth, Texas) where he worked until March of this year. He is now a consultant with Vectech Pharmaceutical Consultants, Inc.

Dr. Sutton has been involved with the USP Microbiology Subcommittee of Revision since 1993, where he currently serves as Vice-Chairman of the Subcommittee on Analytical Microbiology.

Dr. Sutton also operates an information source on the internet – The Microbiology Network (http://www.microbiol.org) that provides services to microbiology related user's groups and supports and Email list devoted to pharmaceutical microbiology (http://www.microbiol.org/PMFList_info.htm).

The USP Proposed Chapter "<1117> Microbiological Good Laboratory Practices"

Scott Sutton, PhD
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Vectech Pharmaceutical Consultants

Introduction

The USP Committee of Experts – Analytical Microbiology (no kidding, that is its name; it will be abbreviated as AMB for the rest of this discussion) has been wrestling with a conundrum for several years. The choice is framed by conflicting expectations of the role of USP chapters and the practice of pharmaceutical microbiology. On the one hand, are the established, large companies with well-entrenched microbiological practices. Their earnest desire, as a rule, is for USP and all other regulatory agencies to remain constant and not meddle in business decisions. This is completely understandable. On the other hand, are the small companies who might be a bit uncertain about how to adequately support the microbiological quality of their products. These companies have repeatedly requested more information as to how microbiology should be conducted in the pharmaceutical environment. Taken to the logical extension, USP would become a manual for laboratory practices.

Let's make it clear from the outset that USP is not going to become a laboratory manual. That is not its charter. However, there is a need to set some base level of microbiological laboratory standards. There, after several rounds of discussion (stretching over a couple years), the AMB has presented a draft chapter entitled "<1117> Good Microbiological Laboratory Practices" (*Pharmacopeial Forum* 29(3):842-850 May/June 2003). The response to this proposal has been spirited, as some of the large pharma companies are commenting that it is too much detail, some small pharma companies are concerned that there isn't enough detail, and, as an extra bonus, FDA staff has expressed concern over whether USP is straying into the realm of GMPs. While this draft is far from its finished form, the AMB seems to be on the right track.

Chapter Organization

The chapter is meant to provide minimum guidance for microbiological practices in the pharmaceutical environment. As such, it is organized into topics of importance to the microbiologist:

- Media Preparation and Quality Control
- Maintenance of Microbiological Cultures
- Maintenance of Laboratory Equipment
- Laboratory Layout and Operations
- Training of Personnel
- Documentation and Maintenance of Laboratory Records
- Interpretation of Assay Results

Let's examine each of these in turn.

Media Preparation

The quality of work in a microbiological laboratory depends on the quality of the culture media. It is essential to use the correct media for the purpose at hand, although the correct media is not always obvious. For example, water testing is commonly done with R2A agar, but many facilities use TSA for

(Continued from page 2)

this purpose. The recommendation is provided that the choice of media should be consistent, appropriate and justified.

The proposed chapter spends some time on media preparation as well. The recommendations include accurate weighing of dehydrated components, the use of high quality water, completely dissolving the dehydrated media or individual ingredients, and the need to control the heating of the media to avoid damaging heat-labile components of the media. Some recommendations on the labeling and packaging of media are also provided.

The quality control of the media is a critical concern. Interestingly, some of the most strident commentary on the chapter deals with the "excessive" amount of space provided to media quality checks. These checks include confirming that the new batch of media adequately supports growth of indicator organisms (as well as a suggestion to control the experiment by comparison to a previous batch). One change from what is apparently current practice in many facilities is the recommendation to move away from streaking the media to demonstrate the nutritive properties and move towards a more quantitative approach where appropriate to be more consistent with the upcoming, harmonized Microbial Limits Tests (see *Pharmacopeial Forum* 29(5) pp 1714-1735 Sept/Oct 2003 for the Stage 4 proposed <61> "Microbial Limits Tests" , <62> Microbiological Procedure for Absences of Objectionable Organisms" , and <1111> "Microbiological Attributes of Nonsterile Pharmacopeial Articles").

Maintenance of Microbiological Cultures

Second only to media, safeguarding the stock cultures is the most important component of a successful microbiology laboratory. These must be handled carefully at all times to avoid contamination.

The care of the cultures starts upon receipt. A careful stock culture curator will confirm the identity of the received cultures, even if they come from as respected a source as a national culture collection. Mistakes happen to everyone, and shipment of the wrong sample to the pharmaceutical lab only means a lost sale to the culture collection, it could imperil weeks of work to the microbiology lab.

This draft chapter provides some detail on the "seed lot technique" for maintaining a culture. Critical to this is the need to go into your containers of stock culture only once, and in restricting the number of passages. Now, it must be stated that there is nothing magic about the number 5. I recently heard an amusing story that this number came from observation in the vaccine industry that viral inoculums could not be passed more than five times through eggs and give a reliable titer of vaccine. While it has justification in vaccine manufacture, its only utility in stock culture maintenance is to prevent a lab from transferring the same slant for years. Whether or not this story is true is irrelevant – the point is that a careful lab will go to great lengths to safeguard the purity and identity of their stock cultures. This chapter is meant to provide guidance on how to accomplish this with minimal effort.

Maintenance of Laboratory Equipment

This section was included more for the sake of completeness than because of concerns peculiar to the microbiology laboratory. Most lab equipment in the microbiology laboratory is subject to the standard validation practices of IQ, OQ, and PQ. As is common, periodic calibration/maintenance may be required for the particular equipment based on its nature, and performance verification checks should also be performed regularly. The frequency will depend on characteristics and use of the equipment.

Laboratory Layout and Operations

The need for this section stems from the concern that too few facilities understand or plan for the separation of samples from a microbiological perspective. The success of a laboratory can be enhanced by the thoughtful separation of samples likely to have contamination from those that are expected to be sterile.

Many responders have become distracted by a perceived requirement for separate entry and exit doors. In an ideal situation, work and traffic in the lab could be completely controlled to avoid bottlenecks and congestion. Few of us work in the ideal situation and it is to be hoped that everyone involved recognizes

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this elementary fact. The description in the text is only meant to provide working directions to stimulate thought and a framework for analyzing the laboratory layout. However your lab is built, it would be well worth your while to document the work flow and the justification for that flow.

Training of Personnel

Probably the most controversial portion of this section is the recommendation that microbiologists and managers in the pharmaceutical support lab should have academic training in microbiology or allied health sciences. The recommendation that the staff and management of the microbiology unit have a basic understanding of, and aptitude for, microbiology is apparently is at odds with some established practices in the field.

In addition to the recommendation that the microbiology staff have studied a relevant subject while in school, the proposed guidance chapter points out a fundamental link between training and the unit's SOP system. It recommends that the SOP system should be comprehensive and serve as basis of the training program. This proposal also recommends that performance assessments be done periodically and should demonstrate competency in core activities of the lab.

There are excellent reviews in the literature dealing in detail with the subject of training in the microbiology lab, and this subject was intentionally left vague in the proposed guidance document. Interested readers are recommended to "PDA Tech Report #35: A proposed training model for the microbiological function in the pharmaceutical industry" which was released in 2001.

Documentation and Maintenance of Laboratory Records

Like the section on equipment maintenance, this section was included only for the sake of completeness.

Interpretation of Assay Results

This section was one of the most hotly debated during the AMB's initial drafting. It was initially entitled "OOS Investigations" it was renamed "Microbial Data Deviation Investigations" out of deference to the work underway by a PDA task force. However, during the writing process it became clear that the scope of this section was broader than merely investigations, and so the current title was settled on almost by the process of elimination.

A discussion of the inherent variability of microbiological data was necessary in this chapter. One view of good laboratory practices could be structured around determining practices that minimize variability in the microbiology lab. However, because we are dealing with such low numbers on plates (frequently less than 20 CFU/plate) and the real opportunities for human error in tests that may run over a month to completion, the microbiologist must always be aware of the role that random chance has in the data and be on guard against over-interpreting the results of a study.

This section of the proposed guidance document is intended to be both a discussion of the limitations of compendial test methodologies and a guide to developing methods of investigating test failures. It discusses the difference between a test that has failed, a test that should be invalidated and a test that should be repeated for confirmation.

Summary

The proposed chapter <1117> "Good Microbiological Laboratory Practices" which appeared as a Pharmacopeial Preview in *Pharmacopeial Forum* 29 (3):842-850 May-June 2003 has generated a good deal of input to the USP. This proposed chapter was developed in response to repeated requests from industry for guidance in this area. While not in its final form, this proposed chapter does go a long way to describe minimally acceptable practices in the pharmaceutical microbiology laboratory.

PMF THREAD

Question 1
The USP draft **<1117> Good Laboratory Practices** (Pharm Preview in Pharmacopeial Forum Sept-Oct 2003 vol29 no5 pp842-850) suggests that all "clean" samples be processed separately, preferably in another room, from "dirty" samples. How do you accomplish this separation?

Choices were:
a. separate room
b. Separate area of the laboratory
c. Clean room
d. Isolator
e. No need to separate these samples
f. Other (please describe)

Answers were:
Answer 1; Clean hood
Answer 2: separate room
Answer 3: separate area in the laboratory and clean hood
Answer 4: No need to separate these samples

Question 2
Since the number of CFU from the EM samples from Class A and B areas should be zero, do you sanitize the exterior of the containers prior to entering the clean area (i.e. do you treat them as you would for materials for sterility test)?

Answers were:
Answer 1: Yes
Answer 2: Yes
Answer 3; Yes
Answer 4: No and Yes

Question 3
How are swabs handled, once resuspended, to plate the sample?

Choices were:
a. Filtered on a manifold, then the filter plated
b. Filtered through Milliflex and then the filter plated

Answers were:
Answer 1: a or b
Answer 2: a
Answer 3: e; swabs directly plated on agar.
Answer 4: b

Question 4
Are the plates and air samples from Grade A and B areas incubated in "clean", dedicated incubators once returned to the lab?
Choices were:
a. yes
b. no

Answers were:
Answer 1: No
Answer 2: No
Answer 3: Yes
Answer 4: No

Question 5
What are the incubation temperatures used for the environmental monitoring samples?

Choices were:
20-25°C or ?? days then 30°C-35°C for ?? days; then read
30-35°C for ?? days then 20°C-25°C for ?? days; then read
30-35°c for ?? days; then read
20-25°C for ?? days; then read
Other (please describe)

Answers were:
Answer 1
30-35°C for 5-7 days; then read (TSA)
20-25°C for 5-7 days; then read (SDA)

Answer 2
23°C for 5 days and then 33°C for 2 days

Answer 3
30-35°C for 3 days; then read: all contact plates at this temperature
20-25°C for 5 days; then read: Two plates taken for each air sample location, one at 20-25 and one at 30-35°C

Answer 4
30-35°C for 2-4 days, then 20-25C for 5 days, then read

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Are you aware of our on-line discussion group? Membership is FREE. To join, send an e-mail to Listserv@peach.ease.lsoft.com. Write ['Subscribe PMFlist' Firstname Lastname] as the first line of text. (message). You can ask, answer, or read questions and comments from your colleagues.

USP Corner

The PMF recommends that you *write directly to the USP with your comments on all proposals*. You can write representing your company, or as an individual scientist.

Any questions concerning USP documents should be sent to David Porter, Ph.D. You can reach Dr. Porter at: (706) 353-4514, via mail at United States Pharmacopeia, 126 Twinbrook Parkway, Rockville, MD 20852 or via e-mail at DAP@USP.org. When communicating with Dr. Porter, let him know you are a PMF member.

Current Compendia

US Pharmacopeia (USP) 27/ National Formulary 22
Supplement 2 effective August 1, 2004

European Pharmacopoeia (EP) 4.0
Supplement 4.4 April, 2003
Supplement 4.5 July, 2003
Supplement 4.6 January, 2004
Supplement 4.7 April, 2004
Supplement 4.8 July, 2004
European Pharmacopoeia (EP) 5.0—01/2005

Japanese Pharmacopoeia (JP) XIV 2001
Supplement 1, 2003

Chinese Pharmacopoeia (1995)

* If you use any other compendia, let us know for inclusion in this corner.

Warning Letters and 483s

June 17, 2004 (483's)

A lack of appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile. [21 CFR 211.113 (b)]

In your responses to the FDA-483 item, you stated that your firm understands the concerns that the FDA investigator raised during the inspection. One of the FDA investigator's major concerns was that your firm has not adequately determined the root cause in the majority of the sterility failures. Some of your investigations found some equipment malfunctions and failures. These are possible introduction pathways for microorganism contamination. Why were the microorganisms not detected in the environmental testing? Your responses indicated that there were no sterility failures since June 2003 and no adverse trends in complaints. The absence of sterility failures and adverse reporting trends do not indicate to us that sterility assurance has been attained. Sterility assurance is achieved by showing the controls and procedures implemented to prevent microbial contamination.

No evaluation has been performed to show the adequacy and efficacy of the cleaning and disinfection process used in parenteral filling room as specified by SOP ____ [21 CFR 211.42(c)(10)(v)].

The environmental monitoring systems in the small volume parenteral manufacturing and filling areas are deficient in that your firm has not performed a scientific assessment to identify appropriate environmental monitoring sampling sites during the actual manufacturing and sterile filling operations that could pose the most microbiological risk to the products manufactured. Inspectional observations include failure to perform air sampling in the area near the vial turntable to assess the condition of the air during manual loading of vials. Environmental monitoring of personnel was not performed immediately after a significant intervention into the Class [redacted] area. Equipment such as forceps, carts, and tools used during the filling operation a was observed being sprayed directly over the [redacted] located in the Class [redacted] area during the media fill. This occurred after intervention through the plastic curtains that surround the Class [redacted] area and after Rodac sampling of the plastic curtains was performed. Environmental monitoring for viable organisms in the manufacturing area is done in the center of the room at times when there is no activity in the room. [21 CFR 211.113(b)]

Article Review

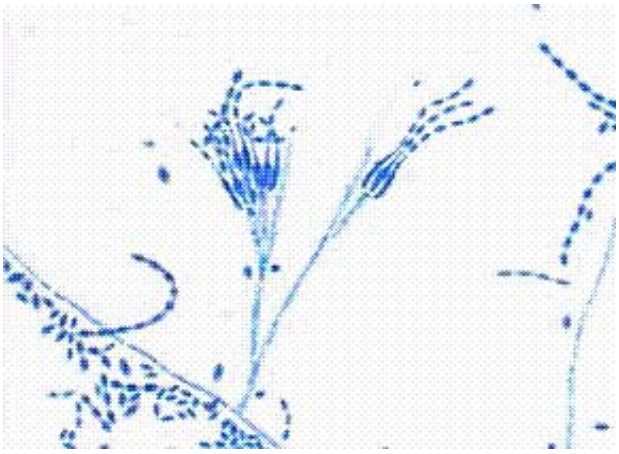
The Importance of Emerging Rapid Methods Technology to Regulators and Industry
Michael S. Korczynski, Ph.D.,
Journal of GXP Compliance, Vol. 7 Number 4.

The author in this article does an excellent job discussing the advantages, difficulties and hurdles that face the pharmaceutical industry and regulatory bodies in adopting rapid methods technology, specifically in the area of microbiology. The FDA has in the past adopted and accepted rapid methods in the area of microbiology; the LAL endotoxin test is an example. The author believes that the way in which the LAL endotoxin test was accepted could serve as the model for implementing other microbiological rapid test methodologies. The author also points out another important initiative that could help the acceptance and use of rapid methods in the area of microbiology; this is FDA's initiative to introduce the concepts of Process Analytical Technologies (PAT) to the pharmaceutical industry and to include rapid microbial methods in this PAT initiative. PAT will "provide an opportunity to move from end product testing and its associated documentation, to a system of continuous quality assurance, by testing or measuring at in-process stages or at-process stages." The advantages of rapid microbiological testing ideally would be increased efficiency, productivity and ultimately cost reduction.

The author also goes into detail on points to consider for rapid microbial methods based on three categories: quantitative detection, qualitative detection and characterization of microorganisms. He then details currently available technologies that could be used for each category. Recently, there has been an increase in new specialized rapid microbial methods. Switching to any of these new rapid methods from conventional methods requires not only a cost analysis to determine financial feasibility but also consideration of method validation issues and regulatory inspectors that would be evaluating these methods. The author points out that validation of a new rapid method may not yield results similar to a conventional method and consequently may not be an easy task. This coupled with the need to give field inspectors guidance on how to evaluate data from the new method may be too daunting for many pharmaceutical firms.

The author does make it clear that the FDA is working with industry to establish some guidelines that will hopefully facilitate the future technical transfer and use of microbiological rapid methods in the pharmaceutical industry.

Fungal ID Tips
Paecilomyces



Morphology: Wooly to powdery, white, beige, pink, lilac orange brown to rust colored.
Hyphae: Hyaline, smooth.
Sporulating Structures: Conidiophores simple to branched. Phialides thin and elongate at tips, grouped in brush like clusters.
Conidia: oval fusoid in long chains.
Paecilomyces resembles *Penicillium* and can be differentiated by its phialides which have thin elongate tips, often slightly spayed apart.

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THIS APPLICATION IS:

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To Update my information, as indicated	
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Pharmaceutical Microbiology Forum
C/o Laura Valdes-Mora
3166 Wood Valley Road
Panama City, FL 32405

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