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PMF NEWSLETTER

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to the continued success of the *PMF Newsletter*

Interesting Times

Scott Sutton, Ph.D.
Editor, PMF Newsletter

We have the pleasure (or as some would have been cursed) with living in interesting times. As the compendia work towards harmonization, everyone is scrambling to see if all non-sterile product tests need to be revalidated. As the FDA introduces cGMPs for the 21st Century, a large portion of their promise requires the use of new methods in microbiology, and calls into question well-accepted practice.

The *PMF Newsletter* has published a series of articles examining the new Microbial Limits Tests, and the difference between the compendial requirements for "Absence of Specified Microorganisms" and the FDA concern over "Absence of Objectionable Microorganisms." This issue of the *Newsletter* includes a short essay on how a company might determine whether an organism found in their non-sterile product was indeed objectionable.

In terms of new methods, sometimes the only facet of the test that is "alternate" is a degree of automation. In fact some new methods are nothing more than highly automated versions of existing methods. This situation has encouraged David Jones to discuss the potential of expanding USP chapter <16> on automated methods to explicitly include microbiological methods.

The month of June saw the debut of a new PMF conference devoted to GMP in the Microbiology Lab. This is an important topic in the current regulatory climate, and the conference was a lively and informative one - it is reviewed beginning on page 4.

Finally, I heard a good story recently from Ranaud Jonquières who has allowed its presentation in this issue. It is about three monkeys and their corporate banana policy. Enough of that, enjoy the story and enjoy the summer. We will be working on the Bacterial Endotoxin Summit and the Fall Forum - hope to see you there!

Scott Sutton, Ph.D.
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How to Determine if an Organism is "Objectionable"

Scott Sutton, Ph.D.
Vectech Pharmaceutical Consultants, Inc.

Previous articles in this series (1, 2, 3) have examined the difference in focus between the USP/NF requirements for the absence of specific microorganisms in monograph products and that the USP Microbial Limits Tests does not address the legal requirement that non-sterile products be free of "objectionable organisms" as set forth in 21CFR 211.113 and 21 CFR 211.165.

So, since the microbial limits tests do not address themselves to "objectionable" microorganisms, how is the manufacturer to determine if there are "objectionables" in a lot of product awaiting release? One approach is suggested by FDA - once all organisms grown in the total count studies (total aerobic as well as total yeast and mold) are identified, a qualified microbiologist would conduct a risk analysis on the presence of that organism in that medication (4).

This risk analysis should incorporate a minimum of four separate analyses:

- Absolute numbers of organisms seen
- Microorganism's Characteristics
- Product Characteristics
- Potential Impact on Patients

Absolute number of organisms seen

Although high numbers of non-pathogenic organisms may not pose a health hazard, they may affect product efficacy and/or physical / chemical stability. An unusually high number of organisms seen in the product may also indicate a problem during the manufacturing process, or an issue with a raw material. The high bacterial counts may indicate that the microorganisms are thriving in the product. If a preserved product this could indicate that the product's preservative system is not functioning or worse, the preservative was missing or incorrectly formulated.

The Characteristics of the Microorganism

The characteristics of the microorganism can be determined by a search of textbooks, or library work, by internet searches, or a combination of all of these. It is always a good idea to remember that you are interested in the microbiology of the situation – do not restrict the search to pharmaceutical sources as most of the best information will come from food, environmental, clinical and perhaps cosmetic microbiology sources in addition to the pharmaceutical field.

During this search look for synonyms of the organisms current name. With the widespread use of genetic techniques in taxonomy the names of some organisms have undergone multiple changes. The national culture collections are a good source of synonyms and all name variants should be researched.

First of all, determine if the organism is a known pathogen. A good place to start on this search is the FDA web site the "Bad Bug Book." This is only a guide, but a good one (5). One approach is to do a preliminary evaluation for any organism that appears on the FDA/CFSAN list and immediately classify that organism as "objectionable." However, it is also important to consider the route of administration and the susceptible population in this evaluation.

A second characteristic of the microorganism that must be taken into account is the potential for the organism to cause spoilage of the product. Make a list of substances used by the microorganism for growth. This can be from the literature, or from the identification equipment. For example, the Vitek 2 Compact will provide an

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Is the Time Right to Include Microbiology in USP Chapter <16> Automated Methods of Analysis?

David Jones, Ph.D.
Genomic Profiling Systems

USP Chapter <16> "Automated Methods of Analysis" is very useful to the chemistry community in providing a means to qualify new automation methods without engendering the full burden of a complete qualification/validation process as described in USP Chapter <1225>. The chapter provides examples of several tests that are amenable to automation in the chemistry laboratory.

Current Pharmacopeial tests detect and enumerate microbes that replicate in the presence of microbiological media. Several automated microbiological tests rely on the same principle to detect and enumerate the same types of microbes. An example of an automated presence/absence test for detecting microbes would be the BacT/Alert system from bioMérieux. There are also systems that automate detection and enumeration of cells replicating to form colonies on plates containing nutrient media. Examples of these technologies are the QCount from Spiral Biotech, the ProtoCol from MicroBiology International (MI) and the Growth Direct™ System from Genomic Profiling Systems (GPS).

With the draft chapter "<1223> Validation of Alternative Microbiological Methods" going through review (1) the question arises: is the time is right to expand the scope of chapter <16> to include automated microbiological tests?

There is a need to distinguish between "automated compendial" tests and "alternative" tests, as they require different validation approaches. Automated compendial tests differ from alternative microbiological tests in that the automated tests are based on the same methods and principles and measure the same targets as the manual compendial tests. Alternative tests, on the other hand, use distinct methods and principles and measure distinct targets e.g. ATP bioluminescence, "Fluorescent events" *etc*. compared to compendial tests.

Automated compendial tests differ from the manual compendial tests in that some manipulations and/or detection steps are automated. For example, colony counting by GPS' Growth Direct System and MIs ProtoCol uses the same method principles and measures the same colonies as do the tests described in several USP chapters. Both the manual and automated approaches enumerate colonies derived from microbes that can replicate on a media support. The automated system, however, uses digital imaging to detect the colonies, in contrast to the manual method in which colonies are detected by eye. The automated imaging is more reproducible and allows faster enumeration times.

Alternative tests use different method principles and do not necessarily measure the same quantities as the compendial tests. For example, the AES Chemunex ScanRDI method measures the numbers of cells showing esterase enzymatic activity rather than the number of colony forming units – the quantity measured by the compendial methods. Consequently, the targets measured by the ScanRDI system can be very different than those measured by the compendial tests, since not all of cells with esterase activity can replicate in the presence of microbiological media.

For alternative tests, validation must be concerned with demonstrating that measuring different targets leads to equivalent or better results compared to the compendial method. The proposed USP Chapter <1223> recommends validation strategies for alternative tests. In contrast, for automated compendial microbiological tests – since the targets measured are identical to those measured by the Pharmacopeia methods – validation should be focused only on demonstrating that the automated aspects

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Internet Address	Description
Wikipedia http://en.wikipedia.org/wiki/Main_Page	A free encyclopedia that is open to all for editing. This may be the purest example of the truism that Truth is whatever most people believe at a particular moment.
Suite101.com http://www.suite101.com/article.cfm/microbiology/118545	A site containing monthly articles on microbiology topics geared to the layperson without significant training.
If you have found an Internet site that contains information of relevance to pharmaceutical microbiology, please let us know.	

The 2006 PMF Conference on GMPs in Microbiology

Scott Sutton, Ph.D.
PMF

A new conference made it appearance in June of this year with the PMF-sponsored meeting "GMP for the Microbiology Laboratory" held in Philadelphia, PA on June 5th and 6th. This conference featured speakers from the United States Pharmacopeia, the American Society for Quality and the United States Food and Drug Administration in a lively and active two-day interactive meeting.

Richard Friedman of the FDA led the meeting off with a discussion of the Agency's initiative "GMPs for the 21st Century" and their impact on the microbiology function, a function central to the monitoring of the state of process control, finished product release and validation of the manufacturing process. This presentation was followed by Don Singer (American Society for Quality and USP) who reviewed the regulatory basis of testing, and the role of microbiology and appropriate validation studies in the development of meaningful product specifications. At this point the conference became a generalized discussion amongst the speakers and registrants on a variety of topics that lasted well into the afternoon. Rick Friedman and Scott Sutton ended the day with a discussion of the microbiology lab in particular, reviewing the FDA guidance on inspection of QC pharmaceutical microbiological laboratories and the ISO document "ISO 17025 General Requirements for the Competence of Testing and Calibration Laboratories" which has some very useful insight into running an effective laboratory. The first day ended with a mixer featuring a local jazz guitarist.

The second day began with a review of the upcoming USP chapter "<1117> Best Microbiological Laboratory Practices" which will be effective August 1, 2006. The group then discussed different tools for risk assessment, and in an FMEA exercise applied those tools to real-life examples. The second day finished up with a discussion of current methods for microbial identification and an extremely animated discussion on all topics covered by the conference.

The conference speakers:
Scott Sutton, Richard Friedman and Don Singer (L-R)

Comments from attendees:

"Thoroughly enjoyed the conference. Learned a lot and met great people. Very friendly and lively format."

"This PMF conference has been the most informative conference that I have attended in the past several years. I hope to be able to attend this conference yearly going forward. Keeping current on FDA expectations and bench marking with other companies' procedures is instrumental to running a successful pharmaceutical microbiology laboratory. Thanks for a great conference!"

"Overall, the conference was very informative and the speakers very knowledgeable. It is helpful to interact with the participants."

"It would have been helpful to know where in the handout binder the latest presentation was located." (*Ed. note: there was some confusion about presentation handouts which will be addressed in future PMF conferences*).

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The Three Monkeys

A cautionary tale as told by Renaud Jonquières of Biomèrieux

It seems that there was at one time three monkeys locked in a room. This room also contained a banana, suspended by a cord from the ceiling in the center of the room. Whenever one of the monkeys grabbed the banana, all the monkeys were subjected to an icy cold shower. After several repetitions of this shower, no monkey in the room would touch the banana.

One of the monkeys was then removed and a new monkey added in his place. The new monkey, of course, saw the banana and moved towards it. The other two set upon him with violence, preventing him from getting the banana. The new monkey quickly learned that it was not permitted to touch the banana.

A second of the original monkeys was then removed, and a new monkey replaced him. The newest monkey attempted to get the banana, but the other two forcefully taught him the "banana policy." Soon he also knew that the banana must not be touched. Finally the last of the original monkeys was removed and replaced by a new monkey, who was also taught the wisdom of avoiding the banana.

We now have a room with a golden, succulent banana suspended in the center from the room's ceiling, and three monkeys. None of the three monkeys has ever been subjected to the icy cold shower, but all three will enthusiastically enforce the "banana policy" even though they have no idea why it exists or if it still useful.

Don't miss the opportunity!

Discuss the LAL Test
PMF Meeting " Bacterial Endotoxin Summit"
September 11-12
San Francisco Bay Area, CA.

Speakers:

- Karen McCullough, Roche Molecular Systems, *MODERATOR*
- Michael E. Dawson, Ph.D., Associates of Cape Cod, Inc.
- Alan Baines, Cambrex Bio Science Walkersville, Inc.
- Foster Jordan, Charles River Endosafe
- Ronald N. Berzofsky, Ph.D., GeneChoice, consultant
- Robert Mello, Ph.D. FDA

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"Please consider giving seminars for personnel working in the Micro lab w/o any theoretical background but just laboratory experience. VERY GOOD CONFERENCE."

"Very good conference. Would like more training courses, more basic like performing MLT or Sterility Testing. All speakers were very good & relaxed format was a good environment for learning & sharing information."

"Lots of great information. Presentations were on a level appropriate for beginners & veterans."

This conference was very well received, and in the opinion of PMF a needed addition to the scientific discussion. With the inclusion of several new microbiology chapters into USP at the second supplement to USP 29 (effective August 1, 2006), more emphasis will be placed on the functioning of the microbiology laboratory and the role of microbiology in monitoring and documenting product quality.

PMF is proud to be leading the effort to bring the role and importance of the microbiology function in Quality Control into proper perspective. This conference will be presented again, and please feel free to contribute ideas and concerns.

As always, PMF is grateful to the conference planning company High Peaks Associates who did a fantastic job with the marketing, logistics and execution of this meeting.

Upcoming Events

August

- 23rd - 25th **Environmental Monitoring**
  Location: Washington, DC
  WebSite: http://www.ivthome.com/shop/scripts/prodView.asp?idProduct=1824

September

- 11th - 12th **PMF Bacterial Endotoxin**
  Location: San Francisco Bay Area, CA
  Web Site: http://www.highpeaks.us/2006/BES/

October

- 2nd - 3rd **PMF Fall Forum**
  Location:
  Website:

- 27th - 28th **PDA Microbiology Meeting**
  Location: Amsterdam, The Netherlands
  Web Site: http://www.ivthome.com/shop/Scripts/prodList.asp?idcategory=2&sortField=STARTDATE

Offering In-House Courses on Microbiology/ Aseptic Processing:

- The Microbiology Network/High Peaks Associates http://www.highpeaks.us/in-house.htm
  - GMP for Microbiology Labs
  - Validation of Microbiological Methods
  - Water Systems
  - Basic Microbiology for Manufacturing Personnel

- USP
  Contact Steven Paul (stp@usp.org) for information on the course "Fundamentals of Microbiological Testing"

USP Corner

Any questions concerning USP documents should be sent to Radhakrishna (Radha) Tirumalai, Ph.D. You can reach Dr. Tirumalai at: (706) 353-4514, via mail at United States Pharmacopeia, 126 Twinbrook Parkway, Rockville, MD 20852 or via e-mail at RST@USP.org. You can write representing your company, or as an individual scientist.

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of the test deliver equivalent accuracy and precision.

In conclusion, the technologies available to the microbiologist are moving forwards to enable automation to be installed in the laboratory and alleviate some of the manual processes involved in traditional microbiology. In that respect the progress in microbiology is akin to the chemistry laboratory when their analyses were automated. As a result, we suggest that the time is right to facilitate the implementation of automated methods through inclusion of Microbiological methods into the USP <Chapter 16>.

References

1. USP. 2002. <1223>Validation of Alternative Microbiological Methods (Pharm Preview). *Pharm Forum* 28(1):154-160
2. USP. 2003. <1223> Validation of Alternative Microbiological Methods (In-Process Revision). *Pharm Forum* 29(1):256-264
3. USP. 2005. <1223> Validation of Alternative Microbiological Methods (In-Process Revision). *Pharm Forum* 31(5):1475-1486

Discussion List Update

PMFList:
Number of Subscribers: 1,646
Number of Countries: 62
Number of Messages Last Month: 290

PSDGList (Pharma Stability Discussion Group):
Number of Subscribers: 806
Number of Countries: 19

Membership is FREE. To **join the PMFList**, visit http://microbiol.org/pmflist.htm and register.

A sister Email is devoted to topics in the **stability testing** of pharmaceuticals, medical devices and personal products. To **join the PSDGList**, visit http://microbiol.org/psdglist.htm and register.

You can ask, answer, or read questions and comments from your colleagues. Archives of the lists are available at:

- http://lists.microbiol.org/archives/PMFLIST.html
- http://lists.microbiol.org/archives/PSDGLIST.html

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extensive list of compounds the microorganism can metabolize, the Biolog a list of carbohydrates utilized, *etc*. Use the information gained during the identification of the organism. Compare these to the product formulation for potential issues. A microorganism is also objectionable if it has the potential to degrade the product on stability.

Evaluate the microorganism's tolerance to unusual conditions:

- low or high pH
- high salt concentration
- high sugar concentration (osmotic conditions)
- Low water activity
- Growth temperature, *etc*.
-

It can also be useful to determine if the microorganism has a recognized proclivity for harboring plasmid-mediated antibiotic resistance. This is a special concern in regards to horizontal transmission of the trait within an vulnerable patient population.

Product Characteristics

The dosage form is important to consider. Is the product anhydrous or water based? This can have an effect on the ability of microorganisms to proliferate. Does it have sufficient free water to support microbial growth (6, 7).

Is the container designed to minimize contamination and subsequent spoilage? Closure design can have a major effect on in-use stability of a product. Is the container adequately designed to retard access to the environment, and to prevent contamination from the environment. Give special consideration to the likelihood of an anhydrous medication's exposure to water, providing the potential for microbial proliferation.

The route of administration is also important. A medication orally administered can tolerate some microorganisms that would be disastrous in a medication meant to be applied topically to abraded skin or to rashes. Similarly, some microorganisms that could be tolerated in a topical would cause severe distress to a patient if taken orally. Inhalants, although not required to be sterile, are a particularly sensitive area and great care should be taken in classifying *any* contaminate as "non-objectionable."

Other product-related considerations should include a review of the production records and the environmental monitoring trends, A review of field complaints is also useful (is this contaminant one that causes eventual returns?).

Patient Population

Finally, a consideration of the targeted patient population is in order. The manufacturer cannot control, and should be held accountable, for patient abuse of a product or off-label use of the product by physicians. However, reasonable use of the product should be considered and part of the risk analysis.

Are patient populations that are likely to use this product at increased risk if exposed to the particular microorganism?

Summary

The FDA's concern with non-sterile dosage format is that the product not contain "objectionable" organisms. This FDA concern has been made clear since the 1970's. However, many companies continue to mistakenly believe that if their non-sterile product meets the requirements in USP, it will be safe from FDA dispute. This not the case. The manufacturer is responsible for all contents of his drug product. Should question arise over the appropriateness of a particular organism, the manufacturer is expected to have a justification for the presence of that organism, preferably as part of the batch release document.

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Response to "Review of The Parenteral Society and The Scottish Society for Contamination Control's *Risk Management of Contamination (RMC) During Manufacturing Operations in Clean rooms"* by Eric Strauss

Dear Editor,

We wish to respond to the review of our Technical Monograph No 14, 'Risk Management of Contamination (RMC) during Manufacturing Operations in Cleanrooms', published jointly by the Parenteral Society and the Scottish Society for Contamination Control. The review was written by Eric Strauss and published in the 6th of June edition of the *PMF Newsletter*. We offer a number of points of clarification.

Firstly, a reviewer is expected to have a good knowledge of the subject they are reviewing, this being the normal reason they are chosen. However, although we have no doubt as to Eric Strauss' competences in his field of knowledge, by his own admission, the review of the monograph was undertaken 'with an eye to the learning of a (simple?) risk management system'. Starting from this level of knowledge, it is no surprise that the reviewer had difficulty in understanding this Monograph and failed to read it in full. We have spend many years writing, teaching and consulting on Risk Management and Risk Assessment in the field of contamination control and are unhappy that our work has been dismissed so lightly by a reviewer who has limited knowledge of the subject.

The information contained in our Monograph is not 'simple' to understand and will need effort by anyone with a limited knowledge of the subject. It cannot be used in a simple 'cookbook' fashion, and certainly cannot, as the reviewer says, be 'put into use easily'. Correct use of Risk Management Systems is not simple, and it is clear that many people are very uncertain how to apply such systems, and their efforts lead to over simplification and mistakes. For people who wish to obtain an 'easy fix' process that may convince those with limited knowledge that they have an effective (contamination) Risk Management system in place, then our Monograph is not appropriate for them. Those who wish to put effort into learning about the concepts of contamination risk management, and how to correctly apply it, should read the Monograph. The reward for their effort will enable them to derive a suitable risk system for their own cleanroom, that will give the most accurate answer. Indeed, feedback from industry personnel who have adopted this approach has been highly supportive. The choice of system is left to the individual users, but we would be unhappy if they were influenced by Eric Strauss' review.

Bill Whyte, University of Glasgow
Tim Eaton, AstraZeneca

Response from the author:

The author recognizes the value of the approach advocated by Dr. Whyte and Eaton, but at the same time is concerned over the complexity of these analytical methods. The use of the model, as described, requires training and expertise. This training and expertise allows the correct application of a rather intricate series of calculations that results in a numerical value being placed on an analysis formerly performed by experience and intuition, and as such is to be commended and supported. The handicap to the method, and all such methods, is the complexity of the process to reach its conclusion, with the results taken on experience and faith (experimental support for the predictions of any or all risk analysis models of aseptic processing would be welcome in this discussion). This by no means is meant to impede the implementation of the model by trained professionals, merely to note the limitations of the technique for those who are not professional risk analysis practitioners. The PMF Newsletter, after all, is a publication directed at the bench microbiologist, who needs to be aware not only of the tools, but also their appropriate application and the level of expertise required for their implementation.
The author regrets any inference that the risk analysis model advocated by Whyte and Eaton is flawed, but stands but his original position (supported by Whyte and Eaton) that this is a complex analysis that requires specialized training

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Presented here is a brief description of some factors to consider in determining if an organism is objectionable. These considerations include:

- Absolute number of organisms present
- Microorganism characteristics
- Product characteristics
- Patient Population

These are not meant to be a comprehensive listing of all issues, but rather a starting point for the non-sterile manufacturer to use in establishing their program to qualify finished product bioburden.

References:

1. Sutton, S. 2006. The Harmonization of the Microbial Limits Tests; Enumeration. *PMF Newsletter* 12(3):2-3 PDF Copy
2. Sutton, S. 2006. The Harmonization of the Microbial Limits Tests; Absence of Specified Microorganisms. *PMF Newsletter* 12(4):2-8 PDF Copy
3. Sutton, S. 2006. Microbial Limits Tests: The Difference Between "Absence of Objectionable Microorganisms" and "Absence of Specified Microorganisms" *PMF Newsletter* 12(6):3-9 PDF Copy
4. Guide to Inspections of Microbiological Pharmaceutical Quality Control Laboratories. 1993 http://www.fda.gov/ora/inspect_ref/igs/micro.html FDA
5. http://vm.cfsan.fda.gov/~mow/intro.html
6. USP. 2004. <1112> Application of Water Activity Determination to Nonsterile Pharmaceutical Products *Pharm Forum*. 30(5):1709-1713
7. Werner, D. 2000. Water Activity: An Underestimated Parameter in Pharmaceutical Quaity Control. *Pharmeuropa.* 12(3):373-375

The PMF Fall Forum

October 1-2, 2006
Rochester, NY

The Web Survey results are in, and the final planning stages of the 3rd Annual PMF Fall Forum have begun. The presentations will be developed with current leaders in the topics shown to be of the most interest by this survey.

Thanks again for all your input.
PMF

PMF Fall Forum Survey Results	
Desired Topic	**Response**
Microbial Identification	57.6%
Disinfectants	51.5%
Environmental Monitoring Investigations	51.5%
EM for Non-Sterile Manufacturing	47.0%
Investigations of Finished Product Testing	47.0%
Microbial Limits Tests	47.0%
EM Sites	45.5%
EM Analysis	45.5%
Enumeration	42.4%
Biological Indicators	37.9%
EM Methods	37.9%
Training	37.9%
Antimicrobial Effectiveness Test	33.3%
EM for Aseptic Manufacturing	31.8%
EM Software	31.8%
Sterility Test	28.8%
Lab Housekeeping	21.2%
Media Fills	21.2%
Sterilization	21.2%
Container/Closure Testing	19.7%
Isolators	19.7%
Seed Lot Cultures	15.2%
Biosafety	10.6%
Antibiotics	7.6%
Biosafety Containment	6.1%