PMF Newsletter

A quarterly publication of the Pharmaceutical Microbiology Forum

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President's Message

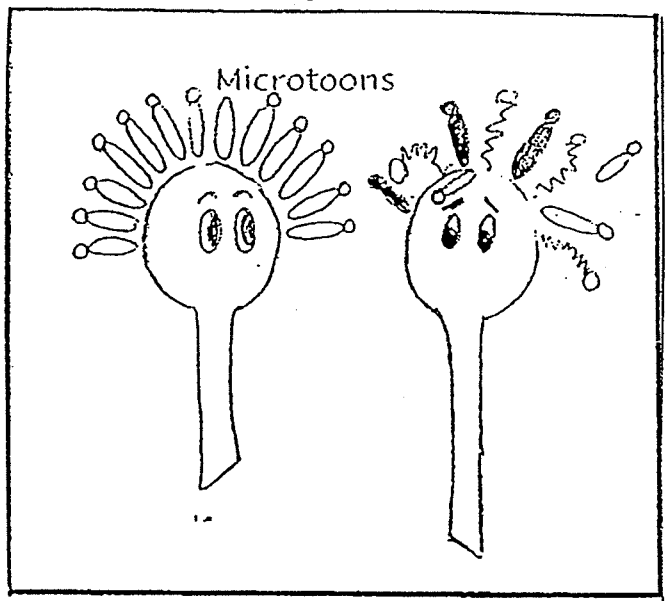
Container Closure Integrity is becoming a popular topic these days. Last year, we published two methods used for ingression testing. However, it seems that pharmaceutical microbiologists and scientists in other areas are searching for additional information. If you have experience in this area, we welcome you to write any information you would like to share as recommendations for this type of test.

I would like to take this opportunity to remind all our members and friends that the PMF is now available electronically. You can obtain information about the organization visiting our worldwide web site at http://microbiol.org/pmf.htm. More detailed information, membership form and on-line membership registration can be obtained via the Microbiology BBS. By joining the Microbiology BBS and PMF On-line, you will be able to read past issues of the PMF newsletter and soon you will be able to post you technical questions electronically. In this manner, PMF's goal is to provide pharmaceutical microbiologists with non-confidential information at a faster pace, in order to be in synchrony with current technology.

If you have posed questions in the newsletter, you know it will take 3 more months to get an answer. Electronically, we hope you can get an answer within a few days and we also expect to have more people responding to questions. For additional information, see page 2 of this newsletter. We now have BBS and PMF available via internet. See you in cyberspace!

MICROTOONS

Below is one of the Microtoons submitted from the Spring Newsletter. Thanks to all of you who submitted them. Please submit any other "toons" you may have to L. Valdes-Mora. We will publish them as space permits.



"I JUST WASHED MY CONIDIOPHORES AND I CAN'T DO A THING WITH THEM."

Inside This Issue

The Microbiology BBS

Scott Sutton

Purpose

The Microbiology BBS was created to facilitate communication among microbiologists in the industrial, food, cosmetics, and academic arenas. It allows the convenient exchange of mail, files, and information among users. This is critically important in these times of international marketing and regulatory issues, with concerns over changing regulatory requirements and new product opportunities arising around the world. The microbiologist must keep up with these changes, and be able to provide state-of-the-art testing capabilities to a wide range of potential markets. Towards this end *The Microbiology BBS* is the on-line home of the Pharmaceutical Microbiology Forum (PMF), an organization devoted to the free exchange of ideas and news about regulatory requirements of interest to microbiologists.

A second major function of this service is to act as a repository for computer files of interest to microbiologists, healthcare practitioners, and interested laypersons. Examples of these files range from programs designed by individual investigators to demonstration programs supplied by vendors. There is also a growing section on international regulations, with PDF files of recent CPMP, and ICH guidelines available. Back issues of the PMF Newsletter are also available to PMF members in the PMF file library.

Finally, *The Microbiology BBS* will serve as a source of information for the interested worker with the development of on-line databases as requested by users or corporate sponsors. These databases can cover upcoming meetings and training opportunities, contract testing facilities, and job opportunities.

Access to the BBS

Access to *The Microbiology BBS* is available by several mechanisms. Traditional telephone access is readily gained by calling 817-557-0330 (N/8/1). *The Microbiology BBS* is also available over the internet-telnet to "microbiol.org" (no quotes). While these communication aveneus can be utilized using standard software, Windows users will prefer using the software provided by the BBS. The software will be mailed to anyone interested free of charge upon request. Email your request to: sysop@microbiol.org, and include your name, affiliation, address and phone number.

The Web Site

The Microbiology BBS has a site on the world wide web at http://microbiol.org. In addition to providing complete information about the contents of the BBS, this page also offers information about microbiology resources. Users groups are invited to take advantage of an offer to establish a free web page to publicize their group. A resource center is provided to direct interested people to contract labs, recruitment services, consultants, and vendors of use to the microbiology community. On-line mail list subscription is provided, and information on the PMF on-line membership is also available at http://microbiol.org/pmf.htm.

The PMF Online Email List

PMF operates a mail list over the Internet for industrial microbiologists in the pharmaceutical, cosmetics, and food industries world-wide. The goal of this mail list is to help the participants share experiences and information on testing methods and requirements. To take advantage of this resource you must have access to Email over the Internet and be a subscriber to PMF Online (available on *The Microbiology BBS*). To participate in the list, send an Email to "PMFList@microbiol.org" with the topic of "Subscribe." This message will be approved if you are a member of PMF Online, and you will be able to communicate via Email with an international collection of working microbiologists. If you have any questions about this service, please do not hesitate to Email "sysop@microbiol.org" for more information, or visit the website at http://microbiol.org/pmfline.htm.

The following is a summary of presentations at the Microbiology seminar, "Microbiology Requirements and Regulatory Compliance in the Pharmaceutical Industry" held in Wilmington, NC in April of this year.

USP Microbial Requirements

The presenter gave an overview of the most commonly used microbiological tests described in the USP. He clarified what is written in the USP versus what industry may do or interpret. The tests are not QC methods. Many of the chapters that are proposed for revisions in the Pharmacopeial Forum (PF) were updated based on the USP Open Conference (see PMF's notes on our previous newsletter). The new proposals will appear in the PF starting (possibly) in the July-August issue.

Antimicrobial Preservative Effectiveness

- The monograph allows for use of any medium as it indicates "media favorable to vigorous growth."
- A plate count of the inoculum is to be performed prior to testing.
- Inoculum requirement is 100,000 to 1,000,000. Currently there is no accuracy statement, therefore 90,000 is not acceptable.
- The requirement that preservative systems do not need to kill fungi is of concern. If a sterile product is contaminated by a mold, the mold will stay. Should preservative systems for sterile products require fungicidal activity?

Microbial Limits Testing (MLT)

- This test is an estimate of microbial content. However, regulatory people consider it an absolute.
- Absence of Pseudomonas aeruginosa does not equal absence of Pseudomonas sp. Problems with Pseudomonas cepacia are occurring and this organism cannot be detected with the Microbial Limits test as described in the USP.
- Sample size of 10mL or 10 grams for each test is up for revision, primarily due to the biotechnology industry.
- Preparatory test indicates that if the organism fails to grow, one should repeat the test until growth is obtained.
- A sample can have S. epidermidis, S. hemolyticus, and S. aureus mutants and pass the test.
- Mutants will not be detected. Example: S. aureus coagulase negative, Pseudomonas aeruginosa with no fluorescence, E. coli colonies with no metallic sheen.
- The problem with mutants is that the test does not require identification of all organisms isolated.
- MLT will just tell you if the product can legally go to market. It will not state if the microbiological quality is good or bad.
- Objectionable organisms will differ based on the type of use of the product (nasal, otic, topical, etc.). How the product will be used will determine which organisms to look for in addition to obtaining a total count.
- Bulk materials are recommended for MLT's.
- The speaker recommended a 3 lot validation with the microbial isolates to eliminate variability in raw material.

(cont. pg. 4)

(USP Microbial Requirements cont.)

Sterility Testing

- The test only indicates that the articles tested meet the requirement for sterility. The test does not mean a lot or batch is sterile.

- Growth promotion is to be performed in duplicate.

- A specification for recovery is needed. Recovery of 75-80% or more will be okay with FDA for now. People are getting 483's on this.

- If you obtain commercially prepared media, you need to know manufacturing date, not just expiration date, as USP states for how long the media can be stored and the frequency of retest for growth promotion. For unsealed containers, storage requirement is NMT 1 month. Therefore, unless you know when the media was made, you cannot determine it's last date of storage.

- Bacteriostasis/Fungistasis (B/F): If growth is not comparable, current USP allows you to change the product to media ratio. The requirement for not less than 1mL or 50mg will not be allowed in the future. The current requirement has no limit on volume of media used. USP will change this to a maximum of 1 liter.

- The table that explains number of containers per medium is usually misunderstood. If the container has a volume of <10mL, the table indicates that number of containers per medium is 20, while sample volume per medium is 1mL. This is interpreted as taking one unit and getting 2mL from it. Unfortunately, this way one will test less sample. For a test that starts by sampling a low number of articles compared to the production run (i.e. 50,000-60,000 vials), this is a concern.

- USP under membrane filtration indicates to rinse the membrane with (3) 100mL portions of Fluid D. Some people use more rinses, or higher volume. USP does not call for these.

- For known bacteriostatic compounds, USP suggests using a hydrophobic edged filter, or cut out the center of the filter. The Steritest system does not satisfy this.

- B/F is expected to be performed with every lot of samples. This is how it is written. FDA does it this way.

<85> Bacterial Endotoxins Test (BET)

- FDA has declared gel clot, chromogenic, and turbidimetric methods to be equivalent.

- Test of RSE and CSE is to be done in the lab in which the test will be performed.

- During the inhibition or enhancement test, the results are compared to the label claim of lysate, not to each other.

- USP is written to perform Inhibition or Enhancement with each sample.

- The BET test is done in duplicate with positive and negative controls. The design of the test helps in determining if there is a change that can affect the results. Even though this can justify not running inhibition/enhancement with every run, the USP is written differently.

- Each monograph has its own specification for BET. The concentration of EU's allowed is based on the human dose per drug. This is why the limits will differ from drug to drug.

(cont. pg 5)

(BET Cont.)

- Inhalants and oral products do not need BET. The reason is that endotoxins are of no concern, as they must be present in these products in the kilogram level to go through the liver and produce fever.

British Pharmacopoeia (BP) Requirements and Regulatory Compliance in the Pharmaceutical Microbiology Laboratory

The speaker talks about current BP requirements. Here are the highlights:

- The BP is published every 5 years. The current edition is the 15th, published in 1993. There are 3 addenda to date.

- The role of the BP is to provide publicly available standards that are applicable during shelf life, to safeguard purchasers and users of medicinal products.

- BP communicates directly with manufacturer via MAIL (Medicinal Act Information Letter). This is published every 3 months, and is very similar to the Pharmacopeial Forum (PF).

Microbiological Tests

Parenterals

1. Sterility Test - not required for terminally sterilized products if you have a validated sterilization cycle.

2. Pyrogen Test

3. Preservative Effectiveness - This test is performed on the development phase as well as stability trials. A minimum of 2 batches are tested; for new drugs, 3 batches are tested. The test is performed at the beginning and end of shelf life.

Microbial Purity Tests

Raw Materials

There are only 13 materials requiring microbial assessments. High risk materials are those of natural origin, and each batch is tested.

The absence of certain organisms varies with the material.

Topical/Respiratory Products

Total Viable Count(TVC)

10^2(bacteria and fungi)

10^1 Enterobacter and other Gram negatives

Absence of P. aeruginosa and S. aureus.

Oral/Rectal Products

TVC 10^3 bacteria

10^2 fungi

Absence of E. coli

Orals with Natural Materials

TVC NMT 10^4 bacteria

NMT 10^2 fungi

NMT 10^2 Enterobacter and other Gram negatives

No E. coli, S. aureus, Salmonella sp. in 10 grams

Herbals and Boiling Water

TVC NMT 10^7 bacteria

NMT 10^5 fungi

NMT 10^2 E. coli

Other herbals

TVC NMT 10^5 bacteria

NMT 10^4 fungi

NMT 10^3 Enterobacter and other Gram negatives

No E. coli, Salmonella sp. in 10 grams

- Bioburden testing is required for products that can support growth.

- MLT's on tablets are not required unless formulation promotes microbial growth.

(cont pg 6)

(BP cont)

Preservative Efficacy

This is not a routine test

- The test requires real time conditions. Beginning and end time points and also enough points in between, usually 1, 3, 6, 9 and 12 months, etc.

- Study must have sufficient time points to verify proposed shelf life.

Validation of Microbial Tests

- There are very few guidelines on validation in the U. K.

- A three lot validation is considered a good idea (by the speaker).

- Use a documented protocol defining standards expected.

- Factors affecting test variability were cited as: maintenance and reproducibility of test strains, quality and performance of media, inactivation procedure (for preservatives or antimicrobials) and effectiveness of recovering stressed cells. This last item can lead to poor reproducibility and conflicting results.

- Validations must be performed whenever there is a change in conditions, such as medium lot, or when 12 months have elapsed from the last validation.

Validation of Total Viable Count

- This is used for products and water. Effectiveness of culture media, validity of counting method (is it sensitive enough for small numbers), sterility of medium and diluent and aseptic performance of test are evaluated.

- Recovery is evaluated and counts must not differ by more than a factor of 10 from the calculated inoculum population.

Medicines Control Agency (MCA) Audits

The MCA is the British equivalent to the US FDA.

- The Microbiology laboratory produces data. The reliability of the data, how it is interpreted and how it is communicated is important. The discussion on the documentation for stock cultures, methods, data, equipment, personnel training and corrective actions was along the same lines as we are required to have for FDA.

Examples of Common Problems

1. Poor practice in control of stocks. Frequent subculturing with periodic replacement from a master culture.

2. Inadequate cleaning of equipment.

3. Poor equipment maintenance.

4. Temperature fluctuations in incubators.

5. Lack of detailed log books.

6. Poor preparation, dispensing, testing or storage of media.

7. Failure to link sterility failures to manufacturing or media fill problems.

Calendar of Events

The Calendar of Events is provided as a service to PMF Newsletter readers. Submission of complete and accurate information will be published on a space-available basis.

October 21-25, 1996, *Pharmanet Courses,* King of Prussia, Pennsylvania

October 28-November 1, 1996, *PDA Baltimore Course Series (VI),* Baltimore, Maryland

Pharmaceutical Microbiology Forum (PMF) 1996 Organizational Board

President:	Laura Valdes-Mora AAI
Vice-President:	Jeff Werner Biogen
Treasurer:	Betty Darner Ortho Biotech
Assistant Treasurer:	Cindy McInnis Teacher
Secretary:	Sharon Wood Consultant
Assistant Secretary:	Christine Vojt * J & J Clinical Diagnostics

*New Director

QUESTIONS/QUESTIONS

Please send your reply to the following questions to L. Valdes-Mora at the address listed in the newsletter:

1. Does your company use sodium thiosulfate to remove chlorine from RO water systems? What do you do for control of bacterial contamination in the sulfite solution?

2. How were your limits set?

Advertisements:

The PMF newsletter will accept advertisements for both those seeking employment, as well as those with current job openings. We also encourage any advertisements for products or items that are new and of interest to microbiologists. These are published free of charge, at the discretion of the organizational board. Please send these to Laura Valdes-Mora.

Regulatory Corner

Points of Interest from a recent Center for Biologics Evaluation and Research (CBER) Inspection:

- CBER agents were very interested in reviewing water and environmental monitoring data. They reviewed every individual action level excursion and the response.

- The main focus of the inspection was a paperwork review of Batch production records and a tour of the facility. CBER was very interested in sample flow and chain of custody. However, there was little interest in observing any assay work.

- CBER Required expiration dating to be based on real time stability data only.

- They requested that coliform testing be performed on at least one purified drop.

- CBER stated that they expected all WFI drops to be tested once during the same week. Bioburden and endotoxin were a must, but USP chemistry needs to be done on only the worst case drop. They were not as concerned about the frequency of testing purified water.

- They stated that the Agency expects all validation work to be conducted as 3 runs and not less.

- When an upgrade to a validated purified system was performed to the distribution loop, they wanted 5 consecutive days of full testing to be performed. After that period, they were comfortable with a return to a normal testing schedule provided all tests were acceptable during the 5 day period.

(cont pg. 8)

(Regulatory Corner cont.)

European Medicines Evaluation Administration (EMEA) Inspection

•In contrast, they were much less inclined to review paperwork. Instead, they focused on touring of the facilities and watching operations. They were very interested in speaking with and questioning the operators to determine their knowledge.

•They did not review water data, and only spoke about Environmental Monitoring data with the operators who were performing the testing.

•They were very interested in observing the sterility testing operation. In particular, they wanted to look inside the gowning room while a test was ongoing. This was refused and one inspector repeatedly asked to see it. When finally allowed at the end of testing, he "raced" to see the gowning SOP. He was interested in spatial separation of clean vs. dirty side of the room, as well as the uniforms employed.

•They were interested in magnehelic gauges and whether analysts checked or recorded these readings prior to performing the testing. Also, they wanted to know if this was addressed in an SOP, along with acceptable specifications, so that the analyst knew before doing the test that all was OK.

•Additionally, they mentioned that media fills of 3000 units do not have an alert level and the action level is set at 1 unit.

•Finally, they asked to see lab services agreements for all in-process and finished product testing contract labs. Particular emphasis was placed on what the contract lab policy was regarding out of specification results and whether they notified the sponsor before repeating testing.

Future Topics

The purpose of the Newsletter is a sharing of information among Microbiologists. Your contributions to *PMF Newsletter* are needed in the form of short articles, letters to the Editor, job openings, comments, or suggestions. Please direct your correspondence to *PMF Newsletter*, c/o L. Valdes-Mora, 3166 Wood Valley Road, Panama City, FL 32405 [Tel (904) 763-5453]. Submit any articles with your name and phone number in case we need to contact you. Your name and company will not appear without prior written authorization.

USED EQUIPMENT FOR SALE

Used Microbiology Laboratory equipment, such as Biolog, Laminar Flow benches, Milli-Q systems, autoclave, and pH meters are available for sale.

For information contact:

Bing Miller
Fermtech, Inc.
Mt. Airy, MD
(410) 875-2968

PMF Newsletter is published quarterly by the Pharmaceutical Microbiology Forum.

PURPOSE: To provide a forum for discussion of microbiology issues in the pharmaceutical industry.

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MICROBIOLOGICAL BENCHMARKING SURVEY

Mary Connor, AAI's Quality Control Auditor, prepared and conducted a survey at the Microbiology Seminar held in Wilmington, NC this past April. The PMF expresses sincere thanks to Ms. Connor for authorizing the publication of this survey. Here are the practices of 39 pharmaceutical microbiology laboratories across the USA.

SECTION 1. GENERAL OPERATIONS

Has your lab validated melt cycles for agar?

# Responses	% YES	% NO
39	33%	67%

Do you test the moisture content of your dehydrated media?

# Responses	% YES	% NO
38	0%	100%

Do you apply the supplier's expiration date to dehydrated media? (If no, please explain)

# Responses	% YES	% NO
37	95%	5%*

* Based on date opened

You do growth promotion on:

62% every lot of dehydrated or purchased prepared media
31% every receipt of dehydrated or purchased prepared media
8% every container of dehydrated media
69% every autoclaved batch of in-house prepared media
(39 Total # Responses) - Total exceeds 100% because of multiple responses per person

What quantity of media do you growth promote?

# Responses	1 unit	10 ml	100ml	1%	4-5%	10%
23	61%	9%	4%	4%	13%	9%

How many replicates do you growth promote?

# Responses	1	2	3+
19	38%	45%	17%

How many CFUs do you use for general (not sterility) growth promotion?

86% 10 to 100 CFU
5% 30 to 300 CFU
8% low-level
(37 Total # Responses)

Do you quantify the # of CFU used for growth promotion?

# Responses	% YES	% NO
39	77%	23%

SECTION 1. GENERAL OPERATIONS (continued)

What storage condition do you use for your growth promotion suspensions?

# Responses	2 - 8C	RT	FRESH
31	87%	6%	6%

What shelf-life do you assign your growth promotion suspensions?

# Responses	Fresh or Quanticult	1 day	5-7 d	1-2 mo	3 mo	6mo	until non-viable
33	33%	12%	18%	12%	9%	6%	9%

Have you temperature mapped your incubators?

# Responses	% YES	% NO
37	65%	35%

Have you temperature mapped your LAL water bath(s) or heat block(s)?

# Responses	% YES	% NO
23	57%	43%

If yes to either of the above 2, are these mapping studies periodically repeated?

# Responses	% YES	% NO
25	68%	32%

Did you map: just the empty chamber / or mimic load configuration(s) for the incubators/waterbaths? (Circle)

# Responses	Empty only	Loaded
15	44%	56%

What testing do you perform on Biological Indicators?

- 56% Maintain the COA
- 33% Purity assessment
- 61% Population verification
- 42% Gram stain
- 22% Species identifications
- 31% D-value verification
- 06% No testing required

(36 Total # Responses) - Total exceeds 100% because of multiple responses per person

SECTION 1. GENERAL OPERATIONS (continued)

What accuracy does your lab associate with USP incubation temperatures? (Include decimals)

Range	Absolute	+1 Decimal	±0.1 to 0.2C	±0.5C	±1C	±2C	±2.5C	# Resp.
20-25C	15% (20-25C)	11% (20.0-25.0C)	7%	15%	11%	30%	11%	27
30-35C	8% (30-35C)	13% (30.0-35.0C)	8%	17%	8%	33%	13%	24

Temp	±0.5C	±1C	±2C	# Responses
35C	33%	40%	27%	15

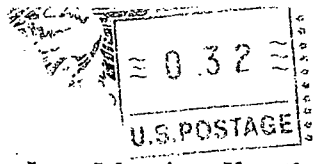
Are your laboratory alert/action levels based on: historical data (or) regulatory literature. (Circle one)

# Responses	History	Regs
36	64%	36%

What accuracy does your lab associate with USP incubation durations?

Duration	±1 hr	±2 hr	±4 hr	±6 hr	±8 hr	±12 hr	±24 hr
48 hr	13%	30%	13%		9%	9%	
18-24 hr	11%	17%		6%	6%		
1 week	6%	12%	6%	12%	18%	6%	12%
72 hr	6%	41%	12%	6%	12%	6%	6%

Duration	Absolute	= 1 day	= 2 day	= 3 day	5 - 7 d	min. of	± 10%
48 hr			9%			9%	4%
18-24 hr	50%	6%					6%
1 week	12%				6%	6%	6%
72 hr				6%			6%



Pharmaceutical Microbiology Forum Membership Application

MISSION: **PMF provides a forum for pharmaceutical microbiologists to exchange information on microbiological issues in the pharmaceutical industry and interact with the USP and regulatory agencies.**

PLEASE PRINT

Name: _____

Title: _____

Department: _____

Company: _____

Address: _____

_____

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Optional:

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Do you have access to E-mail? _____

Please tell us how you heard about us. _____

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c/o Elizabeth Darner
223 Sunnymead Road
Somerville, NJ 08876

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