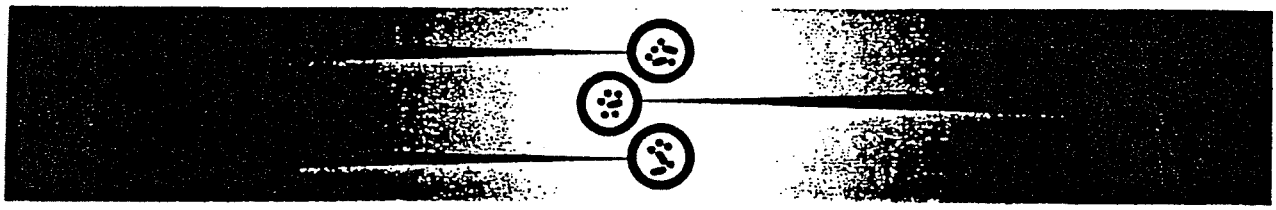


PMF NEWSLETTER

A quarterly publication of the Pharmaceutical Microbiology Forum

DISTRIBUTED INTERNATIONALLY

Volume 6, Number 4

IN THIS ISSUE:

President's Message

The beginning of the 21st century brings a new Millennium. At this point, we look back at what last year meant to our organization. 1999 was the first year we instituted renewal fees and I am pleased to announce that the majority of our members renewed their memberships. We have also received and accepted a record number of new memberships. We have seen the departure of one of our founders from our organizational board and have greeted Lucia Clontz as our newest Director.

We have been able to secure what we referred to as "Feature Articles". These are short articles submitted by various pharmaceutical microbiologists. We thank our latest authors Bob Friedel, Wayne Olson and Tony Cundell for their contributions.

We look forward to having some of our readers submit short articles on their topics of interest. We have a great number of experiences from contamination horror stories, to great discoveries on how to improve a method, to a trick on making a method foolproof or an interesting management challenge anecdote. It is sharing our experiences that we help others prevent some problems. This is the reason why the PMF was founded back in 1992.

As the new millennium unfolds, I invite you to make a lasting contribution to the field of pharmaceutical microbiology. We could be remembered as the first microbiologists of this era. Make history and share your knowledge.

Laura

Changes to USP Microbiology Chapters <61>, <62>, <1111> and <1116>.
By: Dr. Anthony A. Cundell

The following changes were published in the March-April, 1999 Pharmacopeial Forum as Previews: Revisions to USP Microbiological Test Chapter <61> Microbial Limit Tests & General Informational Chapter <1111> Microbiological Attributes of Pharmacopoeial Articles and the addition of New Chapter <62> Microbiological Procedures for Absence of Objectionable Microorganisms.

In addition, the May-June, 1999 Pharmacopeial Forum contained an In-Process Revision to USP General Informational Chapter <1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments to expand the scope of the chapter to include isolators used for aseptic processing, set limits for media fills and recommended alert & action limits for surface, personnel & air monitoring.

- The proposed changes in chapter <61> can be summarized as follows:
- The Microbiological Test Chapter <61> was renamed Microbial Evaluation Tests and contains description of Total Aerobic Microbial Count and Total Combined Yeast & Mold Count.
- A Coliform and Enterobacterial Count was added to harmonize with the EP.
- The methods for objectionable microorganisms, formerly known as USP indicator organisms are moved to Chapter <62> while the numerical Microbial Limits of each dosage form are moved to the General Informational Chapter <1111>.

- In chapter <61> Microbial Evaluation Tests, the expectancy is that a Total Aerobic Microbial Count and a Total Combined Yeast & Mold Count would be conducted on all non-sterile articles following the decision trees contained in Chapter <1111> while the Coliform or Enterobacterial Counts would be performed only if specified in an individual product monograph.

USP chapter <62> Microbiological Procedures for Absence of Objectionable Microorganisms defines a wider range of organisms as objectionable, e.g., *B. cepacia, C. albicans & Clostridium spp.* were added to the USP indicator organisms, and details classical methods to isolate and identify these microorganisms. Unfortunately, the objectionable organisms & methods employed differ from those contained in Eur. Ph. 2. 16. 3 so harmonization is needed. Again, the objectionable organisms are included in the Chapter <1111> decision trees. The additional objectionable microorganisms that are not in Eur. Ph. are *Candida albicans & Burkholderia cepacia.*

The differences between the USP and Eur. Ph. Methods for the isolation and identification of objectionable microorganisms are:

Compendial Test	USP Preview	Eur. Ph. Chapter 2. 6. 13
Absence of *E. coli*	Primary enrichment in Fluid Lactose Broth (FLB), selective enrichment on MacConkey's Agar & confirmatory screening on Levine Eosin -Methylene Blue (EMB) Agar.	Primary enrichment in buffered Soybean-Casein Digest Broth, selective enrichment in MacConkey's Broth and confirmatory screening on MacConkey's Agar.
Absence of *Salmonella* spp.	Primary enrichment in FLB, selective enrichment in Fluid Selenite & Tetrathionate Broths & confirmatory screening on Brilliant Green Agar, Xylose-Lysine Deoxycholate (XLBG) Agar & Hektoen Enteric Agar followed by growth on Triple Sugar-Iron Agar (TSI) slants.	Primary enrichment in buffered Soybean-Casein Digest Broth, selective enrichment in Tetrathionate Bile Brilliant Green Broth & confirmatory screening on Deoxycholate Citrate Agar, Xylose-Lysine, Deoxycholate (XLD) Agar or Brilliant Green, Phenol Red, Lactose, Sucrose Agar followed by growth on Triple Sugar-Iron Agar slants.
Absence of *P. aeruginosa*	Primary enrichment in Fluid Soybean-Casein Digest Broth (FSCDB), selective enrichment on Cetrimide Agar and confirmatory screening on Pseudomonas Agars for the Detection of Fluoresein and Pyocyanin.	Primary enrichment in buffered Soybean-Casein Digest Broth, selective enrichment on Cetrimide Agar and confirmatory screening in Soybean-Casein Digest broth incubated at 41 to 43 C.
Absence of *S. aureus*	Primary enrichment in FSCDB, selective enrichment on Vogel-Johnson, Mannitol Salt and Baird-Parker Agar.	Primary enrichment in buffered Soybean-Casein Digest Broth, selective enrichment on Tetrathionate Bile Brilliant Green broth and confirmatory screening on Baird-Parker Agar.
Absence of *Clostridium* spp.	Selective heat sample to 80 C for 10 minutes to kill vegetative cells, selective enrichment in Reinforced Medium for Clostridium and confirmatory screening in Columbia Agar under anaerobic conditions.	Selective heat sample to 80 C for 10 minutes to kill vegetative cells, selective enrichment in Reinforced Medium for Clostridium and confirmatory screening in Columbia Agar under anaerobic conditions.
Absence of *Candida albicans*	Primary enrichment in FSCDB, selective enrichment on Sabouraud Dextrose Agar and confirmatory screening on Cornmeal and Chlamydospore Agars	No test listed
Absence of *Burkholderia cepacia*	Primary enrichment in FSCDB, selective enrichment on MacConkey's Agar and confirmatory screening on Triple Sugar Iron Agar (TSI) slants.	No test listed

Chapter <1111> Microbiological Attributes of Pharmacopoeial Articles contains guidelines for Microbial Limits which means they are not official USP requirements.

As a result of industry submissions, the fungal limits changed from NMT 10 to NMT 10, 50 or 100 cfu per g depending on the dosage form. A new requirement of a Coliform or Enterobacterial Count was added with a limit of NMT 10 cfu per g when the test is included in individual product monographs as a harmonization step.

An expectancy is that pharmaceutical ingredients with high microbial counts be rendered acceptable for use. Furthermore, composite sampling required in a controlled environment is recommended. More flexibility is possible as the testing frequency may be determined by dosage form. Complex Decision Trees developed based on physical form, intended recipients & water activity specifying microbial testing and objectionable organisms. However, a better approach would be to develop Decision Trees for each dosage form with even more emphasis placed on Water Activity.

Possible changes to the chapter include:

- Eliminate *B. cepacia* & *C. albicans* as objectionable organisms and allow for individual manufacturers to determine that microorganisms are objectionable in their products as per 21 CFR 211.111 Control of Microbiological Contamination.
- Provide guidelines as to when Coliform or Enterobacterial Counts and absence of Clostridium spp. are added to a USP Product Monograph.
- Harmonize testing procedures with the Eur. Ph.
- Reference BAM methods.
- Place greater emphasis on alternate methods.
- Products with Water Activities below 0.75 should not require routine testing.

A question is why would the USP place special emphasis on *C. albicans* and *B. cepacia*. 1997 USPHS/IDSA (US Public Health Service/Infectious Diseases Society of America) Guidelines for the Prevention of Opportunistic Infections in Persons Infected with Human Immunodeficiency Virus is a source document that is useful in setting Microbial Limit specifications for pharmaceutical products. The document states that *Candida* organisms are so common on mucosal surfaces and skin that no measures are available to reduce exposure of immunocompromised individuals to these fungi. Typically, solid dosage forms recalled for fungal contamination have been classified as class III recalls.

The frequency that products, especially oral liquids & inhalation solutions, contamination with *Burkholderia cepacia* occurs in product recalls is a concern. However, this bacterium would not proliferate in products with Water Activities below 0.90. For aqueous products of higher water activities, this organism could be added to the challenge organisms in the USP chapter <51> Antimicrobial Effectiveness Test and not be included as an objectionable organism in USP chapters <62> & <1111>. According to 21 CFR 211.111 Control of Microbiological Contamination, the primary responsibility for the identification of objectionable microorganisms for each dosage form and individual product must lie with the pharmaceutical manufacturer.

With a proposed limit of NMT 10 coliforms per g or mL, we would question that the coliform or enterobacterial enumeration is sufficiently precise to make decisions to the compendial quality of a material. Furthermore, the presence of coliforms in compendial articles does not necessarily imply recent fecal contamination or that pathogens are present in the products. Perhaps a screen for Fecal Coliforms/*E. coli* would be preferable to Total Coliforms. Also, it should be noted that the coliform and enterobacterial counts are not equivalent so a product could pass one test and fail the other.

The table below lists all the members of the family *Enterobacteriacae* that are detected by coliform screening, whether they are of fecal origin, enteropathogenic and can be considered objectionable in oral dosage forms.

Enterobacteriaceae by Genus	Coliforms	Fecal Origin	Enteropathogenic	Objectionable in oral dosage forms
Citrobacter	Yes	Limited strains*	No	No
Edwardsiella	No	Yes	Limited serotypes**	No
Enterobacter	Yes	Limited strains*	No	No
Erwinia	No	Limited strains*	No	No
Escherichia	Yes	Yes	Limited serotypes**	Yes
Hafnia	No	Limited strains*	No	No
Klebsiella	Yes	Limited strains*	Limited serotypes**	No
Proteus	No	Limited strains*	Limited serotypes**	No
Salmonella	No	Yes	Yes	Yes
Serratia	No	No	No	No
Shigella	No	Yes	Yes	Yes
Yersinia	No	Yes	Limited serotypes**	Yes

* Limited strains of fecal origin but many may be associated with plants, water, soil, etc
** Limited serotypes may contain enteropathogenic strains

Although the Pharmacopeial Forum May-June, 1999 In-Process Revision to USP Chapter <1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments was slated for the First Supplement to USP 24, action on the Revision will be delayed. This action was the result of a PDA summary presentation to the August 11, 1999 USP Microbiology Subcommittee meeting. The PDA position was published in the September 1999 PDA Letter.

The following points can be made:

- The informational chapter is proposing target, alert and action cleanliness guidelines for air, surfaces and personnel that are not technically justified as these limits are typically determined from the operational and testing histories from our aseptic processing areas.

- It is recommended that the USP Microbiology Subcommittee invites individuals with experience with isolators to publish a stimuli article to promote discussion of the performance guidelines for isolators.

- The airflow and number of air changes should not be mandated in the USP chapter as product & facility design requirements differ.

- The environmental monitoring limits as stated should be in the Revision, e.g., 85% of the samples must be zero has no technical justification & would be extremely difficult to monitor. There is no technical justification that two consecutive alert levels is equivalent to an action level.

- Since personnel are considered a major source of microorganisms in a well-designed and managed clean room, the importance of temperature and relative humidity control for operator comfort should be further emphasized with recommended ranges, i.e., 20 to 25°C and 30 to 50% Relative Humidity .

- The surface cleanliness level of not more than 10 cfu per contact plate for the floor in a class 100,000 area is totally impractical and this guideline should be eliminated.

- EU GMP, Annex 1 Manufacture of Sterile Medicinal Products has more stringent requirements for air, surface and personnel monitoring of clean rooms during operations. The UK MCA Inspectors insist that air settling plates be employed during aseptic processing & require statistical criteria for Media Fills. Typically U.S. sterile product manufacturers making products for both Europe and the United States are forced to implement the most stringent of the EU GMP and USP guidelines.

- The requirement that the Media Fill contamination rate is NMT 0.1% can be met with 3 turbid vials from a fill totaling 3000 vials. However, to obtain a 95% confidence level that the contamination rate is NMT 0.1% would require no turbid vials from the same media fill.

- The extension of this criterion to three successive media fills is not supported as this would occur at a much lower probability.

ABOUT THE AUTHOR

Dr. Cundell directs a small corporate microbiological development and statistics group supporting all Wyeth-Ayerst Pharmaceutical domestic and international manufacturing sites. Dr. Cundell publishes frequently in the PDA Journal and is Co-Chairman of the PDA Task Force on Evaluation, Validation and Implementation of New Microbiological Testing Methods.

Poster Presentation At The 1999 Annual Meeting Of The American Society For Microbiology (ASM)

Comparison of the Biolog and bioMerieux Vitek Systems for the Identification of Gram Negative Bacteria Encountered in Biopharmaceutical Manufacturing
By: Ed Balkovick, Ph.D.

The Biolog and bioMerieux Vitek Systems were compared for the identification of gram negative bacteria. Three groups of bacteria were tested: 1) 17 ATCC strains of recommended QC organisms, 2) 13 ATCC strains of typical organisms, and 3) 105 unknown isolates submitted for routine identification. Testing was performed in the Biolog System (software version 3.50) using GN microplates and in the Vitek System (software version 6.01) using NFC and/or GNI+ cards. API test strips were used to evaluate discrepant results. The Vitek correctly identified 27 (90%) ATCC strains, while the Biolog identified 23 (77%) ATCC strains in at least 3 separate assays. For the 105 unknown isolates, system acceptable identifications were obtained for 103 (98%) isolates using the Biolog and for 97 (92%) isolates using the Vitek. Both systems yielded the same identification to the species level for 55 (52%) of the unknowns. Comparable results to only the genus level were obtained for 87 (80%) of the unknown isolates. Twenty one (20%) isolates yielded discrepant results. API testing confirmed Vitek results for 2 isolates and Biolog results for 2 isolates. Validation testing was also performed on both systems. The Vitek passed all validation parameters, but the Biolog failed both accuracy and reproducibility. In conclusion, although each system had certain advantages along with some limitations, the Vitek System yielded more consistent results and passed validation testing for the identification of gram negative bacteria from a biopharmaceutical manufacturing environment.

Please direct any questions to:

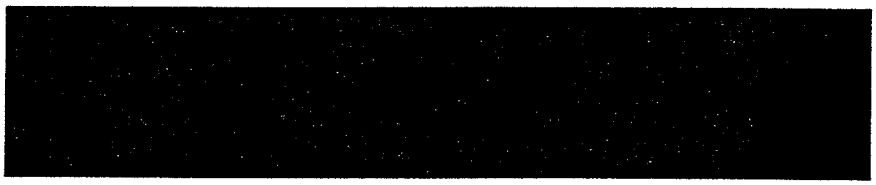
Ed Balkovic
Genzyme Corp.
TEL: 508-473-7584
FAX: 508-872-4091
e-mail: ebalkovic@genzyme.com

ABOUT THE AUTHOR

Dr. Balkovick directs the Microbial Identification and QC Microbiology Technical Support Laboratories at Genzyme Corporation. He is also an Adjunct Professor in the Department of Microbiology at the University of Rhode Island.

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.



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.

Elite MicroSource Microbiology Seminars. Updates, Validation of Lab. Autoclaves & Introduction to Pharmaceutical Microbiology. Dissecting USP <1227>.
-May 17& 18, 2000. Toronto, Canada
-June 28 & 29, 2000. Chicago Area, IL

-May 10, 2000, Sterility Testing in a Barrier Isolator.

-May 11-12, 2000, Identification of Microorganisms using Comparative DNA Sequencing, PDA, Bethsda, MD.

-May 11-12, 2000, Microbiology of Pharmaceutical Waters, PDA, Bethsda, MD.

-May 25-26, 2000, Isolation Technology, Serentec Seminar, Raleigh, NC.

Future Topics

The purpose of the Newsletter is a sharing of information among Microbiologists. Your contributions to the *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 (850) 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.

Current Compendia

US Pharmacopeia (USP) 24 Supplement 1 (January 1, 2000)

European Pharmacopoeia (EP) 1997 / Supplement 2000

Japanese Pharmacopoeia (JP) XIII 1996 / Supplement 1998

Chinese Pharmacopoeia (1995)

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

The following Internet Sites may be of interest to you:

Internet Address	Description
http://www.fda.gov/cder/aerssub/default.html	AER Electronic Submissions
http://www.fda.gov/cder/directories/qi.html	CDER Quick Guide
http://www.comp.uark.edu/~mivey/micro/	Dr. Ivey's General Microbiology Course and Notes
http://www.comp.uark.edu/~wmason/	Jeff Mason's Microbiology Lab Home Page
http://www.cellsalive.com	Information on cells including video
http://www.trishul.sci.gu.edu.au/courses/ss12bmi/microbe_structure.html	Comparative information on Organization and Structure of Microorganisms
http://www.labcompliance.com	Information on electronic signatures, lab compliance, and more
http://www.fda.gov/ora/inspect_ref/igs/high/html	On-line copy of FDA July 1993 Guide to Inspections of High Purity Water Systems
http://www.access.gpo.gov/nara/lsa/lastmth.html	Changes and proposed changes to the CFR during the past month
http://www.vm.cfsan.fda.gov/~dms/fcannex5.html	HACCP Annex 5 Guidelines
http://www.fsis.usda.gov/OA/haccp/imphaccp.html	Index to additional sites in HACCP
http://www.fda/gov/ora/inspect_ref/igs/qsit/qsitguide.html	Guide to Inspections of Quality Systems

If you have found an Internet site that contains information of relevance to pharmaceutical microbiology, please let us know.

Come Visit Our Website at http://www.microbiol.org/PMF.htm

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

REGULATORY CORNER

Examples of 483's:

1. **Incubation of media used for growth promotion testing is inconsistent with the media fill incubation conditions.**
2. **Failure of sterile gowned operators to protect the integrity (sterility) of the sterile gown.**

Word Search

Y	C	F	E	L	A	I	V	K	R	X	G	G	Q	R
T	G	B	S	O	P	F	P	H	B	H	N	Y	A	D
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AUDIT

BIOBURDEN

BUTT

CBER

CIP

COLONY

HVAC

LOGBOOK

MICROBIOLOGY

OOS

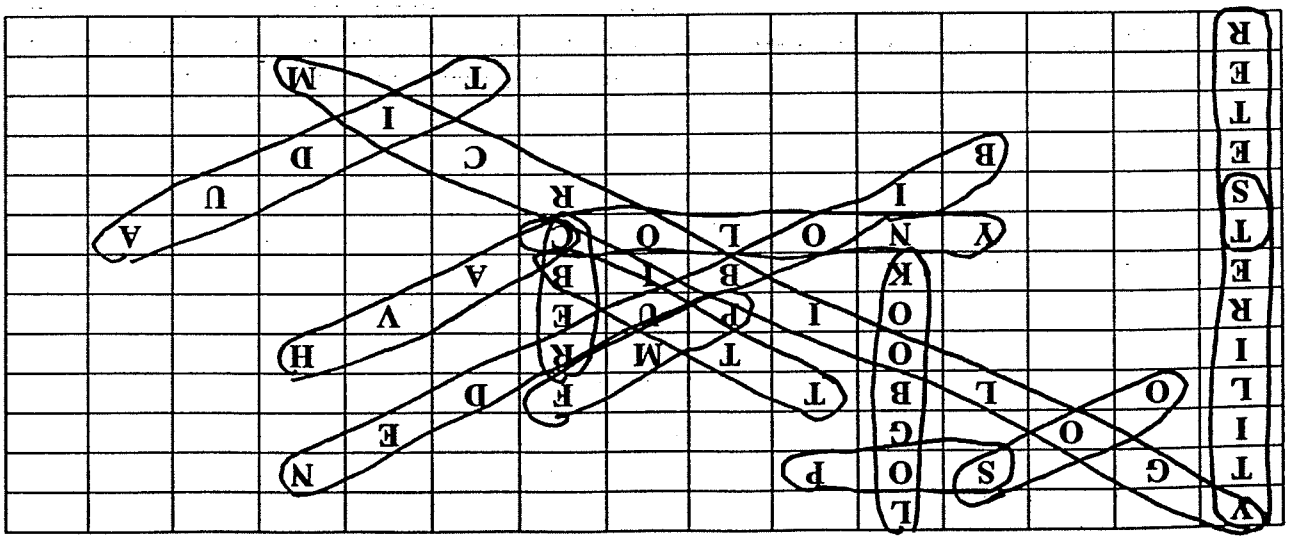
PMF

RETEST

SOP

STERILITY

Word Search Solution



Pharmaceutical Microbiology Forum
Membership Application
or
Change of Information Form

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THIS APPLICATION IS:

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To Update my information, as indicated	
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Please tell us how you heard about us: (add any details below under "Other")

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Another internet site	☐	A PMF member or officer	☐
PMFLIST (an internet news list)	☐	Other (please describe)	

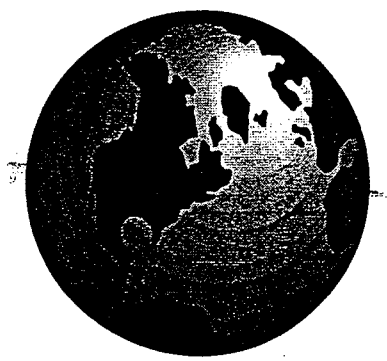
The PMF membership list is private, not for sale.
Membership dues are $15.00. Please send check or money order payable to the 'Pharmaceutical Microbiology Forum' to the address below. Renewal fees are $10.00 only to be paid when announced. Invoices are sent for renewals. PMF EIN number is 56-1874828.

Pharmaceutical Microbiology Forum
Lucia Clontz
c/o Serentec Inc.
612 W. Lane St.
Raleigh, NC 27603

Pharmaceutical Microbiology Forum
c/o L. Valdes-Mora
3166 Wood Valley Road
Panama City, Florida 32405

Address Correction Requested

The Pharmaceutical Microbiology Forum is proud to have members in the following countries: Argentina, Belgium, Canada, Finland, Germany, Israel, Japan, Puerto Rico, The Netherlands, The United Kingdom and The United States.



WE WOULD LIKE TO WISH ALL OF OUR READERS A HAPPY NEW YEAR!!!