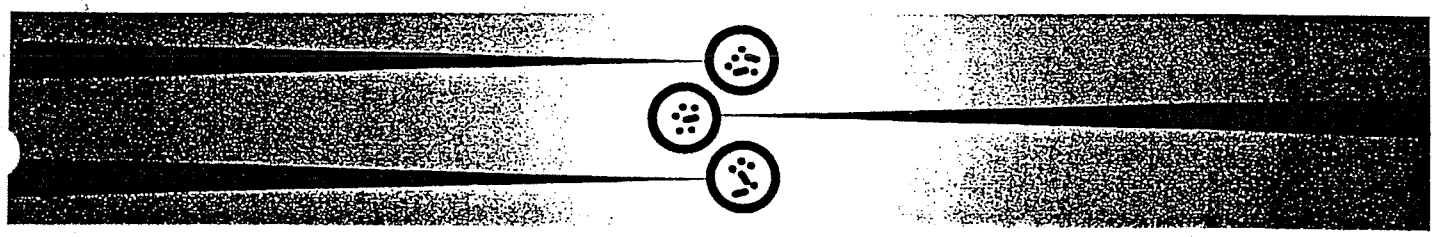

PMF NEWSLETTER

A quarterly publication of the Pharmaceutical Microbiology Forum

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Volume 6, Number 3 Fall 1999

Pharmaceutical Microbiology Forum (PMF) 1999 Organizational Board

IN THIS ISSUE:

PURPOSE: To provide a forum for discussion of microbiology issues in the pharmaceutical and related industry.
The information contained in this newsletter is the professional opinions of our members and does not represent the policies or operations of any corporation or government agency to which members may be associated. *PMF Newsletter* is intended to serve as an open forum and confidentiality will be maintained. The information in *PMF Newsletter* is solely for information purposes and is developed from sources believed to be reliable. Statements expressed constitute current opinions derived through analysis of available information and professional networking. Articles or opinions are for information only for PMF members to stimulate discussion and are not the views of the PMF board or regulatory agencies. The *PMF Newsletter* cannot make any representations as to the accuracy or completeness of the information presented and the publisher cannot be held liable for errors.

President's Message

The controversy regarding the proposals for USP Chapters <61> and <62> continues. Several scientists are diligently working on experiments and on publishing the findings to prove that the proposed methods leave a lot to be desired. Controversy has also started on the current proposal of Chapter <1116> which deals with clean room environments. When I use the word controversy, it means that many people have serious questions regarding the proposed changes and opinions are being voiced all over the country.

Although, we may easily find faults in many of the USP Chapters dealing with Microbiology, we should try to understand their intent.

First the USP is not a textbook. A person who is not a microbiologist cannot pickup a chapter and execute the test as written. This is not the intent of the USP.

Second, any corporation is free to develop and validate a method that proves to be superior to the USP method. Data showing that the new method captures and surpasses the intent of the USP needs to be generated if the company intends to use the new method in lieu of the USP method.

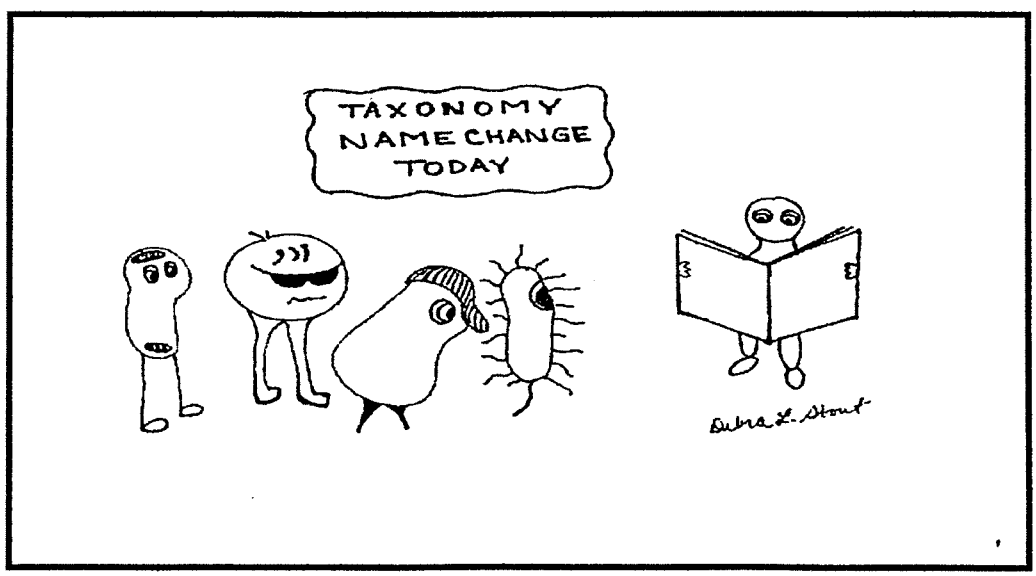
The USP methods are referee methods. These are methods that will be used in case of a legal dispute, usually between a Regulatory Agency such as FDA and the manufacturer. USP methods, for microbiology, tend to be simple methods that are within the reach (easy to set up) of any company group or person, not only in the USA, but in any country in the world.

Yes, I do enjoy the controversies. However, at times, we need to take a step back and try to understand the mission and vision of the USP and their impact across the globe. I would like to extend an invitation to the USP to clarify its mission and vision statements for those of us who work in the microbiology trenches and benches.

Laura

Laura

MicroToon



United States Pharmacopeia—Revisions to the General Information Chapters in Pharmaceutical Microbiology
By: Robert R. Friedel

In the 1999 March-April issue of Pharmacopeial Forum (*Pharmacopeial Previews* section), the USP Microbiological Subcommittee has renamed General Information Chapter <1111>, **"Microbiological Attributes of Nonsterile Pharmacopeial Articles"**. Two very important statements regarding preservation and subsequent microbiological quality are listed in the chapter, including:

1) "*Nonaqueous or dry dosage forms do not support microbial growth due to low water activity. As a result, the microbiological quality is a function of the microorganisms introduced by the raw materials and during processing.*

2) "*The concentration of added antimicrobial preservative can be minimized if the formulation has intrinsic antimicrobial activity (i.e., antimicrobial ingredients, hostile pH, high osmotic pressure and low water activity)*". This has long been recognized by food industry microbiologists and is known as the "hurdle effect".

There are several interesting developments regarding the proposed revision of this informational chapter. The first affects those pharmaceutical articles not covered by individual monographs. In this particular instance, the USP has provided a specific microbiological target of not-more-than (NMT) 1000 cfu/g or mL for raw materials, excipients and drug substances. When the article is not cited as having microbiological specifications, USP has provided a novel decision tree designed to evaluate the need to test for the absence of a particular pathogen. The decision regarding the inclusion of an "objectionable" organism(s) will be based upon the dosage form's route of administration (e.g., Inhalations, vaginal, [nasal, otic or topical], rectal, liquid oral & solid oral), nature of the product (aqueous vs. non-aqueous, multiple-use vs. single-use) and potential hazard to the user (count levels). Of course, there are exceptions to the rule and these are addressed as required.

Traditionally, the microbiologist has applied a somewhat limited number of analytical techniques to evaluate the microbiological profile of a particular substance. Many techniques used today have not changed since the time of Pasteur. In today's world however, all techniques involving the cultivation of microorganisms from materials containing inimical, antimicrobial compounds, whether they be preservatives or antibiotics, contain one common goal—the accurate recovery of indigenous bioburden. Techniques used to achieve this include the addition of a chemical neutralizer, dilution and rinsing (membrane filtration).

In order to ascertain the ability of the method to recover viable microorganisms, the antimicrobial effect of the material undergoing analysis must be neutralized in the recovery medium. This is accomplished by eliminating what is known in the laboratory as "antimicrobial carryover". More importantly, the diluent used to neutralize the antimicrobial (e.g., parabens) must demonstrate a lack of toxicity to the organism(s) the microbiologist is attempting to recover.

The USP/NF Supplement 10 (issued May 15, 1999) contains the new General Information Chapter <1227> * **"Validation of Microbial Recovery from Pharmacopeial Articles"**. This chapter provides the microbiologist with information regarding the conditions and methodologies necessary to ensure accurate recovery of microorganisms from dosage forms containing antimicrobial chemicals. The chapter is applicable to: <61> Microbial Limit Tests, <51> Antimicrobial Effectiveness Testing, and <71> Sterility Tests. The information included in the new chapter was published previously by the American Society for Testing and Materials (ASTM document E1054 "Standard Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic or Preserved Products") and has been present in the industrial microbiological literature for quite some time.

USP is taking the correct steps in providing guidelines for microbiological recovery where none have existed previously or which were limited in scope.

Reference:

Russell, A.D. et al. (1979) "A Review: Microbiological Applications of the Inactivation of Antibiotics and Other Antimicrobial Ingredients", J. Appl. Microbiol, 46:207-245.

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*Editorial note: Supplement 10 contains the incorrect version of <1227>. The correct version is in PF (25) March-April 1999 or see USP 24.

Highlights of the 8th Annual AAI Microbiology Seminar: "Current Issues and Practical Applications" on 20 and 21 April in Newark, NJ.

A.J. McCardell, Qualicon RiboPrinter Microbial Characterization System

- The system is essentially a molecular biology system in a box- basically a 'Southern Blot.'
- A single colony is suspended in buffer to which specific reagents are added and then placed into the instrument. Results are available within 8 hours. It produces a 'genetic fingerprint', which is matched against strains in a reference database to get identification. Additionally, the system allows you to use this fingerprint as a typing tool so that you can compare the organism against isolates that you have previously identified.
- The technology is based on ribosomal RNA genes because RNA is highly conserved (therefore, strong homology) and it is very stable (mutations are usually lethal in the ribosomal region).
- There is less than 4 minutes hands-on time per sample.

- Possible applications include: routinely monitor microbial flora in production facility, address sterility positives by tracking sources of contamination, and communicates to non-microbiologists in a scientific language.
- In response to questions, she stated that although possible it is unlikely that recombinant organisms or plasmids would affect its ability to correctly identify organisms. At the present time there are only 20 genera in the database.

Amy McDaniel, Ph.D. Chemunex ScanRDI Rapid Microbial Enumeration

- The instrument is a combination of direct epifluorescence and flow cytometry and is used to count the number of organisms in a sample. Counts are available within 2 hours of sampling.
- It measures the presence of viable cells only. Viability is determined by non-specific esterase enzyme activity and membrane integrity.
- The sample is filtered through a specific 0.45 micron membrane and labeled laser scans the membrane, using 9000 scan lines employing photomultiplier tubes to look for fluorescence.
- The method can be used for filterable samples only. It detects over 400 microbial strains including bacteria, yeast, and molds with a sensitivity of 1 CFU.
- For non-filterable samples there is another instrument - D-Count- which utilizes similar principles to the ScanRDI.
- It is possible to recover organisms off the membrane after analysis is completed but Ms. McDaniel was not certain how many.
- Correlation of plate counts for water: the ScanRDI recovers higher counts than TSA at 3 days but the same counts as R2A at 14 days with the lower temperature.

Barbara Young, Ph.D, Millipore Corp., MicroStar Rapid Microbial Detection Platform

- The MicroStar system uses ATP bioluminescence to count viable cells. ATP is not produced by dead cells. The system employs a Milliflex device with a unique membrane (sample must be filterable). Results are available within 24 hours.
- Future improvements include the ability to obtain a 1 hour identification using a peptide nucleic acid (PNA) RNA hybridization method and also an enumeration within 2-4 hours.

Sterility Assurance Round Table Discussion Group

- The recent PDA Technical Report on media fills recommended a 0.1% non-statistical based approach for media fills (i.e., not more than 1 /1000 with a 3000 vial size run). The EP requires a 0.1% limit with a 95% confidence limit and therefore 4750 minimum number of vials with only 1 positive vial permitted. Most companies are using the EP approach. It is expected that the revision to the FDA Aseptic Fill Guidelines will include this statistical approach.
- For lot sizes less than 3000 vials, the rule of thumb is to use a media fill that is equal to a minimum of the lot size.
- Incubation temperature for media fills: There was a range among the participants. Some use only 30-35° C whereas others use 20-25° C for one week and then transfer to 30-35° C for the remainder of the 14 days. Some perform growth promotion testing of the media prior to incubation only (stating that USP allows sterility test media to be checked every 3 months), whereas most participants stated that they test the vials at least at

the end of the incubation period. Several did the testing at the beginning and the end. If two temperatures of vial incubation were used, some members stated that the organisms were tested at their optimal growth temperatures (e.g., molds at 20-25°C).

- Frequency of sterility test inspections of canisters: some follow the 3,4, or 5, 7 or 8, and 14 or last day of test approach. Others check daily (in order to catch contamination early on if present) except for weekends or holidays but record only on specific days whereas some checked only at the end of the test.

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 Dr. Roger Dabbah. You can reach Dr. Dabbah at (301) 816-8336, via mail The United States Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD 20852 or via e-mail at RD @ USP.org. When communicating with Dr. Dabbah, let him know you are a PMF member.

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 Seminar. Updates, Validation of Autoclaves & Introduction to Pharmaceutical Microbiology.
-October 20-21, 1999. San Francisco, CA.
-November 17-18, 1999. Fairfield, NJ.
-December 8-9, 1999. Philadelphia, PA.
-January, 2000. San Juan, Puerto Rico.

1999 PDA Annual Meeting
-November 30-December 3, 1999 Washington, DC.

A big "Thank You" is extended to Elizabeth Darner (Betty). Betty one of PMF's founders has donated many years as Treasurer and Board Member to the PMF. Betty has done a fantastic job! The PMF would like to wish Betty the best of luck in her future ventures.

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) 23 Supplement 10 (May 15, 1999)

European Pharmacopoeia (EP) 1997 / Supplement 1999

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://fedbbs.access.gpo.gov	Government Printing Office – documents
http://www.health.gov.au/tga	Australian Therapeutic Goods Administration
http://www.health.gov.au/tga/sterilit.pdf	Australian Guidelines for Sterility Testing
http://www.labcompliance.com	Information on validation and qualification of lab instruments
http://www.ifpma.org/ich5q.html#stability	ICH Stability Guidelines
http://www.phase-technologies.com	Information on Lyophilization

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.

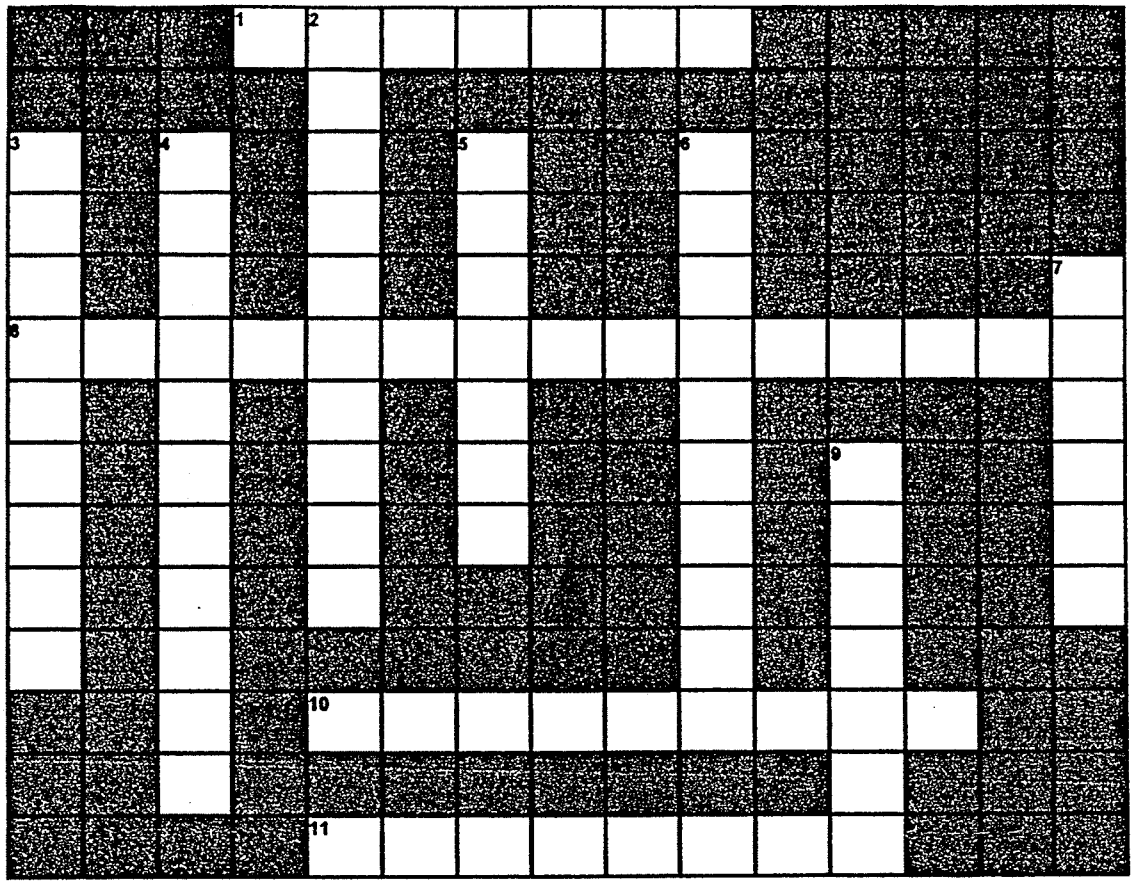
REGULATORY CORNER

Examples of 483's:

1. **Failure to determine that the lowest acceptable level of antimicrobial agent is effective throughout the product's shelf life.**
2. **Lack of sanitizer effectiveness studies.**

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.

Crossword Puzzle



Across

1. hyaline, mucopolysaccharide sheath on the wall of a cell or spore

8. light generated by living organisms (e.g., a firefly)

10. presence of microbes in parenteral tissue

11. type of microscope having two or more lenses

Down

2. a derived pathogen that is avirulent or of low virulence (e.g., often used in vaccines)

3. microbial load

4. capable of growing in the absence of carbon as an energy source

5. spores borne externally in various ways by fungi

6. person, who usually for a fee, provides professional or technical advice

7. a wall, usually a cross-wall, in a hypha

9. having n number of chromosomes

Crossword Puzzle Solution

			D	N	U	O	P	M	O	C				
			I									C		
		N	O	I	T	C	E	F	N	I		I		
			L		N							H		N
M			P		A					D		P		E
U			A		T			A		E		O		D
T			H		L			I		T		R		R
P					U			D		A		T		U
E	C	N	E	C	S	E	N	I	M	U	L	O	I	B
S					N			N		N		T		O
					O			O		E		U		I
					C			C		T		A		B
										T				
					E	L	U	S	P	A	C			

Pharmaceutical Microbiology Forum
Membership Application
or
Change of Information Form

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

THIS APPLICATION IS:

A New Member Application	
To Update my information, as indicated	
Membership Renewal	

Name:
Company:
Department:
Position (title):

WORK (optional) HOME (optional)

Phone:
Fax:
e-mail Address:

Preferred Mailing Address
Address of the above Company ☐ A Home Address ☐ Other ☐
Address
Address
City, State
Country:
Zip

Please tell us how you heard about us: (add any details below under "Other")

Circulated Newsletter	☐	microbiology BBS	☐
www.microbiol.org	☐	An Associate At Work	☐
Another internet site	☐	A PMF member or officer	☐
PMFLIST (an internet news list)	☐	BETLIST (an internet news list)	☐
PMA 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.

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