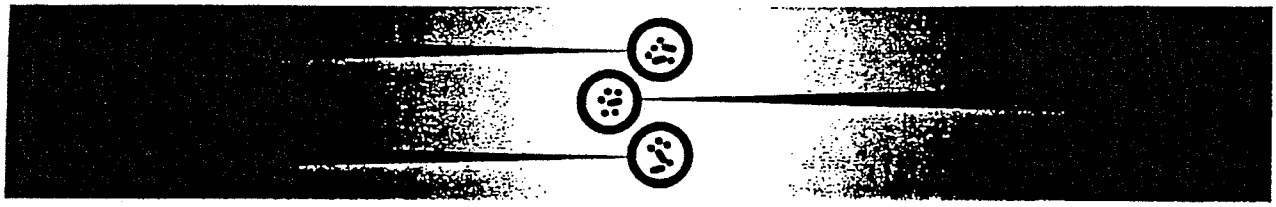


PMF NEWSLETTER

A quarterly publication of the Pharmaceutical Microbiology Forum

DISTRIBUTED INTERNATIONALLY

Volume7, Number 2

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.

Presidents Message

The harmonization effort between the United States Pharmacopeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP) appears to be in high gear and is finally yielding the unified methods we have waited for since the 1990's.

A harmonized method for Bacterial Endotoxin Testing (USP Chapter <85>) is included in USP 24 Supplement 2 with the clarification that the chapter, as written, becomes official on January 1, 2001. The chapter is considered by LAL experts as one that requires careful study since many details have changed including the elimination of the Control Standard Endotoxin (CSE). The Chapter also contains two different Gel-Clot methods: an end point reaction and a quantitative assay.

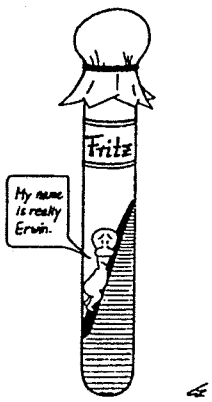
The Sterility Chapter (USP Chapter <71>) has achieved harmonization. The proposal is in the Pharmacopeial Forum (PF) of July-August 2000 and becomes part of USP 24 in Supplement 4, next year. The way the proposal is written, it indicates several differences that will continue to exist between USP, EP and JP. In light of this, then one has to conclude that this harmonized procedure is not truly harmonized and can only be referred to as a "*quasi*-harmonized" method. Hopefully, later on this decade, the differences will be resolved.

Look for a harmonization effort for the Microbial Limit Test USP Chapter <61> in the near future.

Laura

Microbial Identification " A Mystery or an Adventure"

By Ziva Abraham



Bacteria that can endure all the tests for classification are finally given names.

We have all experienced frustrations with microbial identifications, and automated systems do not solve this mystery. Some will not identify certain organisms others are very labor intensive. There are numerous factors that go into choosing the right system for your application namely, cost of the system and supplies, ease of use, completeness of the database and its application to your needs.

WHAT SYSTEMS CAN AND CANNOT DO AND WHY.

Any given system will be able to identify only the organisms it has in its database. Some systems may have a limited database where many species of a given genus are not included. Others may have a broad range of species but many strains for a given species were not included. In addition the genus, species and strains were not tested numerous times to cover all possible metabolic variations. Both culture collection organisms and isolates from many environments are important to provide for the variability of organisms within the environment.

WHAT WE, AS MICROBIOLOGISTS CAN DO TO USE OUR SYSTEM TO FULL POTENTIAL AND BEYOND.

Gram Stain: Although gram staining seems to be the simplest step, it is the most crucial one in correct identification for most identification (ID) systems. This dictates what preliminary biochemical, coagulase, catalase, oxidase, etc., testing should be performed before choosing appropriate card, cassette or system to use for the identification. If this important step is improperly performed we must not be surprised if we see *Salmonella*, *Shigella*, *Vibrio cholera* and other pathogens and non-desirable organisms in our environment, water or product. The decolorization step is the most critical step in the Gram stain and is very technique dependent. Yet, the entire identification is dependent on this procedure.

Medium: Often the ID system vendors will recommend certain growth media and/or growth conditions for

subculture of the microorganisms before they are put into the system. The metabolic profiles that are in the system database are built using these conditions.

If you choose to use medium/conditions other than those recommended the reactions may be different and may not match with reactions of that organism in the library, or could match with a totally different organism. You have the option to optimize "validate" your system, using your choice of media with the organisms from your facility and have your own custom database.

Growth Conditions: The optimal conditions for microbial growth depend on the specific requirement for any given group of organisms. Temperature, humidity, and oxygen requirements are the primary conditions to consider. Environmental isolates typically are recovered on lower temperatures than those for human clinical isolates and will grow at relative room humidity and oxygen requirements.

Many organisms will not grow under routine isolation conditions, those that do usually will grow at ~30° C. But there are some that grow but are not happy. Many environmental isolates are not happy on TSA medium, as it is too rich for their enzyme systems. They grow in tiny colonies and may not grow on subculture. To compound the poor growth problem, the QC labs have multiple tasks and ID is not always the priority, so plates from environmental monitoring (EM) or water monitoring sit in the refrigerator or on the bench until we have the time to attend to them. Some have media dried to resemble a potato chip, others have organisms that have shut down their primary enzyme systems and are struggling to survive.

In a rush to revive them, we transfer them once and then off to the ID systems and we are upset they did not key out in our system. Of course the system is no good, the bugs are crazy and we report out what we get, which is essentially ZILCH. What people say about computers, is true for the microbial ID systems too, 'Garbage in, garbage out'. If the organisms are not given enough time to recuperate from the famine to feast stage then don't expect them to be functioning normally. Organisms taken out of storage should be subcultured at least twice, three times is better.

Mixed Cultures: This is another category of "Garbage in, Garbage out". If the culture is mixed, this will produce mixed biochemical test results and an erroneous microbial profile. When the results are inconclusive, subculture and check for the presence of more than one organism. There are no systems that will tell you that it is a mixed culture.

Supplementary Testing

Supplementary tests are an integral part of identification and are highly recommended as part of the dichotomy. *Staphylococcus aureus* should be confirmed by a positive coagulase test, the oxidase test should be used to indicate the presence of the cytochrome oxidase enzyme and help differentiate the *Pseudomonadaceae* (+) from the *Enterobacteriaceae* (-). The presence of the enzyme catalase is used to differentiate *Streptococcus* (-) from the *Micrococcus /Staphylococcus* (+) groups and *Bacillus* (+) from *Clostridium* (-).

Understanding the metabolic, environmental and nutritional requirements of the organisms found in your facility will enhance your ability to obtain more accurate and reproducible identifications of your isolates and reduce the mysteries to an adventure.

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Credits:

The cartoon illustration was from the book "What's So Funny About Microbiology" by Joachim Czichos, Joachim.Czichos@t-online.de. It is a small book of cartoons about life in the lab and petri dish from the viewpoint of the microbes themselves and the microbiologists who labor with them. Have you asked yourself the following questions: "What are the ups and downs for bacteria on the road to culture?" "What is a microbiologist's greatest fear?" "What do wild type and mutant bacteria do in their spare time?" She has tried to answer these and other puzzling questions in her cartoon book. The book can be purchased on-line from amazon.com.

BI SPORE STRIP VALIDATION

The following discussion was compiled from the PMF List discussion group:

Question: **Does anyone out there verify the spore count from BI strips? And if so, what method do you use?**

Response 1. Whenever we purchase spore strips, we always verify the population since we did have problems with several spore strip lots we purchased. We are now distributing spore strips for Bio-One Sciences and we verify the population and D-value for these as well.

Response 2. USP has a monograph on how to perform this analysis. We perform population and purity assessments by this method.

Response 3. YES-- on every receipt, and again if a certain time passes before use; using the USP method.

Response 4. We validate every lot of BI's that we use in the lab. Pretty much straight enumeration as outlined in the USP for BI-Steam Sterilization carriers. They have recently modified the chapter (USP 24-Supplement 2) as to the requirements for acceptable populations.

Response 5. We daily perform approx. 40 to 50 population verifications on spore strips. The only method we have followed for the past 8 years that I'm aware of is the USP procedure. If followed exactly, results are very satisfactory. In the January 2000 issue of Infection Control Today is an article on 'Population Verification'. This is specific for BI's and has been published in USP 24 Supplement 2. This supplement reintroduces a lower limit for BI population verification. Thank you Dr. Dabbah, thank you, thank you!

Response 6 Regarding Method for BI validation: If you ask three different BI manufacturers what procedure they use, each will likely state they follow USP. If you request a copy of their internal protocol for population analysis you will receive three different procedures. The procedure you ought to follow is the procedure used by the manufacturer for establishing the label claim. Using anything else can lead to large discrepancies and a failure in verifying the stated label claim.

Items of particular importance when trying to reproduce the manufacturers label claim:

1) Media: SCDA or TSA recommended by USP & most BI manufacturers. Brand and even lot used may impact the recover by a log or more. Ask the manufacturer what media they recommend. They should be able to provide you with at least two brands that they have tested. Keep in mind that lot to lot variance does occur as well, and we have rejected lots from approved vendors because that particular lot performed differently with our particular use.

2) Diluent: 3 strips (or discs) into 100 ml of sterile, DI water, and then 1 ml into 9 ml is what USP recommends. Using other diluents (e.g., saline, ringer's solution, etc...) or other dilutions (0.1 ml into 9.9 ml) may be convenient, but saline can reduce the recovery of *B. stearothermophilus*, and other volumes of diluent can introduce differences from how the manufacturer established the label claim. It doesn't save you time if you have to re-do the assay, so it is best to follow the manufacturer's procedure.

3) Maceration of the carrier: Blenders or stomachers work best. Oster makes a household blender that works quite well with Ball canning jars and replacement blades. Save the Blender pitcher for the office holiday party.

4) Agar: freshly prepared or melted? Was it melted at the media manufacturer's recommended temperature (usually about 80° C) or was it placed in the 100 degree water bath you use for heat shocking the *B. stearothermophilus*? At 100° C for more than 1/2 hour the media could caramelize leading to lower counts.

5) Time frame: After the heat shock tube is iced (not just immersed in cool water), the spores should have access to media within about 20 to 30 minutes. Longer time frames can lead to poor spore recovery. Don't pour the plates too thin. If they start to curl before 48 hours then you need to be pouring them thicker.

6) If your testing results in low counts for multiple lots of BIs, but they are consistently low proportionally), look first for differences between your procedure and that recommended by the manufacturer before blaming the BI.

7) Storage: Don't refrigerate or freeze if the manufacturer recommends room temp and RH. Don't store in your desk if the manufacturer says to freeze.

8) Make sure you vortex the dilution tubes very well!! You should be able to see the vortex reach the bottom of the tube during the procedure. If you don't see a vortex then you are probably just shaking the heck out of the tube and not thoroughly mixing the solution.

Response 7 USP 24 second supplement <1035> Biological Indicators For Sterilization states that the user needs only to observe the D value of the indicator from the manufacturers. Although, it also states a user may elect to do the D value determination if they have the capability. My questions are: 1) What is the industry standard of verifying the D value of BIs as it stands today? 2) What is your opinion of how industry standard will change, if at all, when the second supplement becomes effective in July 1?

Answers:

1) Industry standard is to verify spore counts via the USP methods on BI's purchased commercially, but NOT to verify manufacturer's D-values unless the BI's are substantially altered for a particular use (i.e., if a spore suspension is purchased to produce BI's on glass, metal, rubber, etc., the D-values would need to be verified on the new configurations).

2) Since VERY few companies or even contract laboratories are equipped with validated, operational retorts or BIER vessels, or the personnel to run them, I doubt that industry practice will change. The "MAY elect to" and "IF they have the capability" are LARGE loopholes, placed there for excellent reasons; unless the user is modifying the BI's for some specialized use, it makes no sense to expend the resources to verify the manufacturer's D-value.

Response 8: We have a steam BIER vessel at our facility, and always confirm D-values. We do the same for all other non-steam BIs by utilizing external contract labs.

Response 9: From what I have gained from visiting numerous companies, most do not have a BIER unit so they at least verify survival/kill. IF the D-Value is fairly accurate, will hit the all survive and all kill. IF these cannot be hit, one can be sure D-Value is not within acceptable accuracy. At least this way the D-Value is being looked at for accuracy.

JOURNAL REVIEW

PDA Journal 54:4 , On the Cause of Performance Variation of Biological Indicator Used for Sterility Assurance by Hideharu Shintani and James Akers.

The authors compared the differences in carrier materials, primary packaging materials and culture media used in the manufacture of commercial BI's. The study used the same carrier material, primary packaging material and culture medium with different spore suspensions supplied from different BI manufacturers. The authors found no significant differences in the BI resistance or population recovery when these factors were the same with all the suspensions.

They suggested that by standardization of the carrier envelope and culture media by BI manufacturers, the variability could be minimized.

Highlights from the AAI 9th Annual Microbiology Seminar (April 10-11-, 2000; Wrightsville Beach, NC)

Scott Sutton, Ph.D., Alcon Laboratories, ' Overview of Pharmaceutical Regulations that Apply to Microbiologists'

- USP Chapter Numbers that are under 1000 are meant as enforceable, regulatory documents. Numbers of 1000 and above are meant to be non-enforceable guidance documents. You need to remind inspectors of this fact if they try to hold you to a specific point in the chapter.
- Expect to see much USP activity on Biological Indicators in the near future as industry has indicated that there is much confusion with the chapters.
- It is difficult to get approval for aseptically produced products in Nordic countries as they prefer terminal sterilization.
- <1222> Terminally Sterilized Pharmaceutical Products- Parametric Release is a new chapter in early draft.
- The Europeans posted on the EMEA web site in March 2000: CPMP/QEP/2431/98 'Concept Paper on the Development of a Committee for Proprietary Medicinal Products (CPMP) Note for Guidance on Parametric Release'.
- <1208> Sterility Testing – Validation of Isolator Systems- New chapter to be published in Supplement 2.
- Although the USP and EP sterility tests are essentially harmonized, there remain some differences:

  Different organisms and incubation times for bacteriostasis/fungistasis

  Different amounts of product and different numbers of units to be tested

  Different product categories

  Incubation conditions for turbid samples: EP requires a subculture to fresh medium at 14 days with an additional 7 days of incubation.

- The Australians have published on their web site [http://www.health.gov/au/tga, TGA News, Sept 1999 page 6] the following manufacturer's responsibility. If one plans to invalidate a contaminated sterility test, using as the sole criterion that it was due to the environment or the operator, then one is **required** to perform microbial identification using a **DNA typing procedure** (Biochemical methods such as the Vitek, Biolog, or MIDI systems will not be accepted as they are not as sensitive.). You must demonstrate that the organism in the environment or from the operator is **identical** to that found in the test.
  - New USP focus on Nutraceuticals: Three new chapters in draft:

    1) <2021> Microbial Enumeration Tests- Nutritional and Dietary Articles

    2) <2022> Microbiological Procedures for Absence of Objectionable Microorganisms in Nutritional and Dietary Articles

    3) <2023> Microbiological Attributes of Non-sterile Nutritional and Dietary Articles

Carmen Wagner, Ph.D., Serentec, 'Hot Spots in FDA Inspections'

- The current top hot spots for FDA inspections are:

  Laboratory controls

  Management of Process and Laboratory Investigation

  Validation

  Commitment Tracking (i.e., did the firm follow through with their FD483 responses?)

  Training

  Documentation Control

- Other areas of concern are:

  SOPs

Expiration Dating for Clinical Supply

Record Keeping and Traceability

Facility Controls

Raw Material Controls

Justification for Limits

Product Development Documentation

Application Integrity

License vs. Actual Process

Reprocessing/Rework

Scale up and post-approval changes

Production and Process Controls

- FDA expectation is that process and lab investigations be closed out at 30 days and if not able to do so, a written memo be placed on file explaining why.

Scott Sutton, Ph.D., Alcon Laboratories, 'Validation of Microbial Recovery, as stated in USP <1227>

- The scope of this chapter is:

  1) Neutralization of preservatives

  2) Accuracy of microbial plate counts

- It was noted many years ago that as one dilutes out a preservative, its effectiveness follows exponential decreases (instead of a 50% dilution results in a 50% loss of effectiveness).
- The plate count accuracy portion of the chapter was intended to apply to any microbiological method and not just the Antimicrobial Effectiveness Test. The historical practice of counting plates containing only 30-300 CFU and more recently, 25-250 CFU is derived from the counting of *E. coli* in the dairy industry. The mathematical formula in the chapters is taken from an ASTM document and is designed to allow the individual user to establish his/her own upper/lower limits for counting plates.
- <XXXX> Validation of Alternate Microbiology Methods: New chapter to be proposed for USP. Although the General Notices section of USP allows one to choose an alternate method as long as it is equivalent or better, it is difficult to establish equivalency since most microbiological methods are historical in nature and was never validated.

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.

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 2. (July 1, 2000)

European Pharmacopoeia (EP) 1997 / Supplement 2001

Japanese Pharmacopoeia (JP) XIII 1996 / Supplement 1998

Chinese Pharmacopoeia (1995)

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

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COURSES

Are you looking for information on training courses and microbiology conventions?
Try these organizations.
American Society for Microbiology (ASM)- www.asm.org
Parenteral Drug Assn. (PDA, Inc.)- www.pda.org)
The Microbiological Control Conference (held in Florida every November
AAI hosts a Pharmaceutical microbiology seminar every April
Elite MicroSource Corporation, two day seminar every month -EMSource@aol.com
More websites to check out for courses:
www.simhq.org
www.aoac.org
www.iest.org
www.aami.org
www.microbiol.org/class.htm
www.cfpa.com
www.barnettinternational.com
www.gmp1st.com
www.iir-ny.com

REGULATORY CORNER

Examples of 1999 Warning Letters issued by the FDA:

1. Water testing specifications, procedures and practices do not assure that quality requirements are met. For example:
   - No microbial analysis is performed on product water from the -----water system
   - An overflow pipe for the still line in the water system is open to the atmosphere without sterile air filtration.
   - A nonfunctional section of pipe off the condenser in the --------water system is reconnected to a line that feeds the still.
2. The routine heat sanitation of the purified water system has not been validated
3. Failure to establish adequate control measures for air filtration systems in that there were no specifications for velocity and pressure drop in the certification of the HEPA filters on the HVAX system. Additionally, there is no data to support the specifications set for total airflow.
4. Integrity testing is not performed for the HEPA filters in rooms 1 and 2.
5. Sampling frequency and sites for environmental monitoring have not been validated.
6. The cleaning procedure for the lyophilizers has not been validated
7. The stopper wash/depyrogenation processing for autoclave has not been completely validated
8. The ----solution used to maintain pH meters is not assigned an expiration date nor replaced at regular intervals. The solution in use was uncovered and had a white residue around the opening of the bottle.

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 and comments from your colleagues.

The following Internet Sites may be of interest to you:

Internet Address	Description

http://www.fda.gov/ora/cpgm/42_006.html	Program Inspection of Plasma Derivatives of Human Origin Guideline- provides FDA thoughts on water, environmental monitoring, and clean steam testing
http://www.fdspharma.com	Pharmaceutical Training Modules
If you have found an Internet site that contains information of relevance to pharmaceutical microbiology, please let us know	

JOB POSTING

A rapidly expanding pharmaceutical R&D and testing contract company has an immediate opening for the following position:

1. Senior Pharmaceutical Development Microbiologist

BS in Microbiology with 5-7 years or an MS in Microbiology with 2-4 years experience in pharmaceutical industry. Must have experience in method development/validation and testing in microbial limit test method, sterility test method, bacterial endotoxin method, antimicrobial preservative effectiveness test and antibiotic bio-assays. Knowledge or exposure for the evaluation of microbiological aspects of sterilization, aseptic filtration and package integrity plus some supervisory experience will be preferred. Excellent verbal and written communications and a full knowledge of applicable GMPs are a must for this position.

Please send resumes to: info@slpharma labs.com. SL Pharma Labs is conveniently located near Wilmington, DE, near I-95.

WORD SEARCH

M	P	P	S	V	C	G	A	E	U	S	Z	L	P	J
U	P	P	E	G	E	C	V	N	B	U	E	Y	P	T
R	I	Q	L	P	I	A	I	G	I	L	F	S	M	I
E	Y	L	D	G	L	L	R	R	O	U	P	O	H	X
S	H	D	Q	C	U	F	U	O	F	M	T	G	G	S
I	R	I	O	C	O	G	L	I	I	I	U	E	K	W
T	I	T	S	B	L	J	E	E	L	L	X	N	P	O
N	U	E	X	Y	I	S	N	I	M	F	E	Y	T	T
A	I	L	I	C	V	T	T	W	A	V	U	X	V	B
V	N	V	U	M	S	Y	N	F	O	V	Y	E	W	E
I	P	I	Y	D	P	W	C	A	A	W	X	Z	U	F

Antibody	**Avirulent**	**Esculin**	**Motility**
Antiserum	**Biofilm**	**Limulus**	
Autoclave	**Cilia**	**Lysogeny**	

SATISFACTION SURVEY

A number of subscribers were randomly selected to receive a survey from the PMF board to determine their level of satisfaction with the PMF organization and newsletter. The following are the results of the survey. The board was pleased with the overall subscriber satisfaction and we will strive to improve the newsletter to further meet the expectations of our readers. Thanks to those who responded.

n=17	YES	SOMEWHAT	NO
Are expectations of PMF being met?	53%	24%	0

RATINGS	IMPORTANT	NOT AS IMPORTANT
Regulatory Corner	88%	6%
Meeting Summaries	82%	16%
Network Opportunities	82%	24%
Rate the newsletter	76%	24%
Feature articles	65%	35%
Job Postings	65%	35%
Surveys	53%	29%

Suggestions for new services included:

- Overview of a microbiological test for pharmaceutical microbiology-this will be beneficial to new QC microbiologists.
- Send Newsletter through online services
- Career guides-information, job postings
- Advice on how to sell microbiology ideas to management
- Keep the newsletter short
- Comparability testing for (especially new) methods

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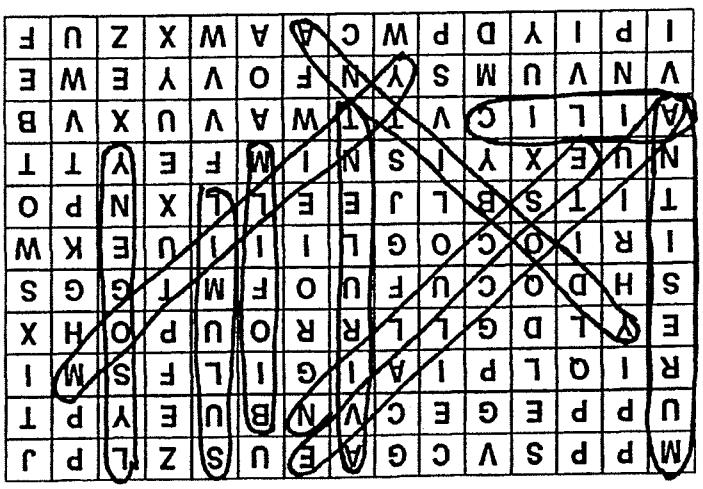
WORD SEARCH SOLUTION



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	☐
www.microbiol.org	☐	An Associate At Work	☐
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.

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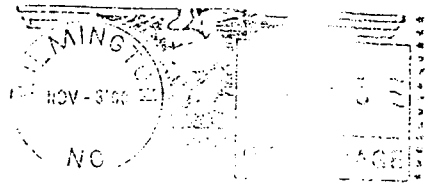

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The Pharmaceutical Microbiology Forum is proud to have members in the following countries: Argentina, Belgium, Canada, Finland, Germany, Israel, Japan, Puerto Rico, The Netherlands, The United Kingdom and The United States.