PMF NEWSLETTER

A PUBLICATION OF THE PHARMACEUTICAL MICROBIOLOGY FORUM
Distributed Internationally to 7,770 Subscribers in 85 Countries

Volume 13, Number 7 July, 2007

CFU on a Filter

An interesting and often overlooked question is how to validate the ability of membrane filtration to remove an antimicrobial substance, especially when this filter will then be placed on an agar surface. The assumption in this method is that nutrients from the agar will migrate through the filter to feed the microbial colony on the filter surface. This issue of the newsletter carries a re-issued Millipore technical bulletin (with permission) that describes a particularly good study investigating this technique with particular emphasis on the effect of nominal pore size on the growth of the colony forming units.

Important Links:

Information on the PMFList at
http://www.microbiol.org/pmflist.htm

Past Issues of the *PMF Newsletter* at
http://www.microbiologyforum.org/news.htm

Another article in this issue reviews some recently proposed revisions to USP chapters that have appeared in the last few issues of *Pharmacopeial Forum*. Keeping abreast of this journal is extremely important as this is the primary method of communication from USP to industry. There have been several unfortunate examples of miscommunication between USP and industry recently - dramatic examples of these being the confusion over the Microbial Limits Tests and the unfortunate errors in the chapter on Antiseptics and Disinfectants (<1072>). This issue of the Newsletter carries some of the germane draft chapters for notification. If these effect your work, by all means review the entire USP proposal and provide comments to USP.

PMF is sponsoring a conference in September on Cosmetic Microbiology!

A new set of pages can be found in this issue. The first of these is a listing (with web links) of recent news items of potential interest. The second is a listing of job openings. If this is worthwhile, we can continue to have them in the newsletter—let us know what you want to see!

Scott Sutton scott.sutton@microbiol.org

Recent Announcements of Interest in *Pharmacopeial Forum*

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

The recent editions of *Pharmacopeial Forum* have carried several articles of interest to the microbiology community. This article will summarize the changes proposed for some of these chapters of interest. Each proposed change to a chapter in USP is preceded by a short segment known as a briefing note. This descriptive text is provided by USP to put the changes proposed in context. The briefing notes will be quoted to describe the changes where appropriate.

It is important for all of us to be aware of the changes under consideration by USP. Of course, it is also important to be aware of changes imminent in relevant documents from EDQM, EMEA, JP, and ISO. But this is especially important for USP as it is the only standard setting body we can affect as an individual. The process of revision employed by USP assumes participation by industry. A chapter is published, and feedback anticipated by the committee. When no feedback arrives from the community, the USP interprets this as an endorsement of the changes (*very* few letters are received congratulating the committee for a good job). While it is tempting to blame USP for recent problems in some chapters, a dispassionate analysis of the situation will include noting the failure of industry to hold up our end of the bargain. Although not the topic of this article, some examples are close at hand in the Microbial Limits chapters (available for review since 2003, no significant comments until 2006) or the Antiseptics and Disinfectants chapter (also available since 2003 and the subject of a withering review only recently). Pointing out problems after the chapter becomes official is not the most effective strategy to ensure a good result.

If there are comments or concerns about these chapter change summaries, please review the text in the cited *Pharmacopeial Forum*. If you, as an individual, have a comment that should be brought to the attention of the USP, then by all means send it in. the most effective commentary is to point out the problem, then recommend a solution or improved text. Commentary that says "this chapter stinks" is entertaining, but will not be given undue consideration.

Contact information is provided in other parts of this newsletter, but the Microbiology USP staff liaison is Radha Timuralai (rst@usp.org).

With this in mind, let's take a look at some proposed changes.

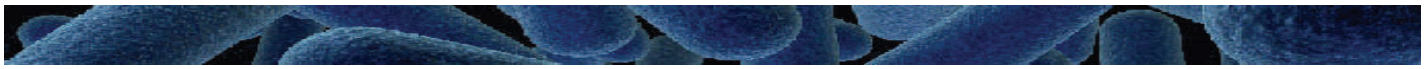
The Microbial Limits Implementation Dates

Official notice of the change in implementation dates came in the March/April 2007 issue of *Pharmacopeial Forum* (1-4). A statement in the "Policies and Announcements"* section of the issue states:

> "These USP-NF General Chapters were originally scheduled to be effective on August 1, 2007. Imple-

* The "Policies and Announcements" section is described by USP as including "information about general scientific and policy issues that may have an impact on *USP-NF* standards and processes and announcements about issues being considered by USP. This section also includes publication and comment schedules."

(Continued on page 5)



Effect of Membrane Filter Pore Size on Microbial Recovery and Colony Morphology*

Summary
Membrane filters with a 0.45 µm pore size have long been recognized as the standard for growth of microorganisms. However, there is little published literature comparing the effects of different pore sizes on colony size and recovery.

The 0.45 µm pore size is used to recover bacteria and other microorganisms from many samples and environments - almost to the exclusion of other pore sizes. Only rarely are other sizes used for growth and recovery and there is little information available on the effects of different pore sizes on microorganisms. However, other pore sizes are commercially available for microbial enumeration and users will occasionally substitute a filter with a pore size larger or smaller than 0.45 µm in an attempt to improve their results.

This study takes a broad look at the influence of different pore sizes on some common microorganisms. It provides data on the effect of pore size on growth and recovery. The study compares a variety of microorganism/media combinations on a range of pore sizes: 0.22, 0.45, 0.7, 0.8, and 1.2 µm. The filtration method used in the study was a standard glass funnel and base with vacuum. Test filters were plated on solid (agar) media and compared against spread plates.

There was no universal pattern of results. Some microorganisms, such as *Micrococcus luteus* and *Candida albicans* showed no significant difference in recovery or colony size with membrane pore size. Other organisms such as *Pantoea agglomerans* showed no difference in colony size but had low recoveries on 1.2 and 0.22 µm membranes.

Acceptable recovery for membrane filters was defined as being 90% versus the controls (spread plates) (1). The 0.45 µm membranes met this definition with all test systems. Some test systems showed equivalent recoveries with other pore sizes but in no case were the results significantly better. The lowest recoveries were seen with extremes of the pore size range (1.2 and 0.22 µm).

Materials and Methods

The pore sizes used in this study were selected from the various sizes of mixed esters of cellulose membranes manufactured by Millipore (Table 1).

The microorganisms and media combinations used in this study were chosen as a broad representation of common membrane filter applications: pharmaceutical, food and beverage, USP testing, water testing, and general microbiology (Table 2).

As an adjunct to the recovery and colony size experiments,

Table 1. Test Filters

Pore Size (µm)	Millipore filter code	Flow Rate (sec/500 mL)	Bubble Point (psi)	Typical Applications
0.22	GSWP	40 to 60	40 to 60	Sterile Filtration
0.45	HAWG	30 to 50	30 to 36	Microbial testing of water, beverages and general microbiology
0.7	HCWG	15 to 23	19 to 23	Fecal coliform testing in surface and wastewater
0.8	AAWG	10 to 16	14 to 18	Yeast and mold testing in beverages
1.2	RAWG	7 to 11	11 to 13	Yeast and mold testing in "hard-to-filter" beverages

Table 2. Test Systems

Microorganism	Source	Media	Temp. (°C)	Time (hours)
Primary effluent	Wastewater	m-Endo LES	35	24
Primary effluent	Wastewater	m-FC	44.5	24
Primary effluent	Wastewater	m-TEC	35-44.5	24
Bacillus subtilis	ATCC 13933	Tryptic soy agar	35	24
Brevundimonas diminuta	ATCC 19146	Tryptic soy agar	30	48
Candida albicans	ATCC 10231	Tryptic soy agar	35	24
*Clostridium sporogenes**	ATCC 11437	Tryptic soy agar	35	48
Enterobacter aerogenes	ATCC 49701	Tryptic soy agar	35	24
Escherichia coli	ATCC 25922	m-FC	44.5	24
Micrococcus luteus	ATCC 9341	Tryptic soy agar	35	24
Pantoea agglomerans	Well water	m-Endo LES	35	48
Pantoea agglomerans	Well water	Tryptic soy agar	35	24

* Originally presented as a Millipore Technical Bulletin this study is reprinted here with permission.

(Continued on page 4)

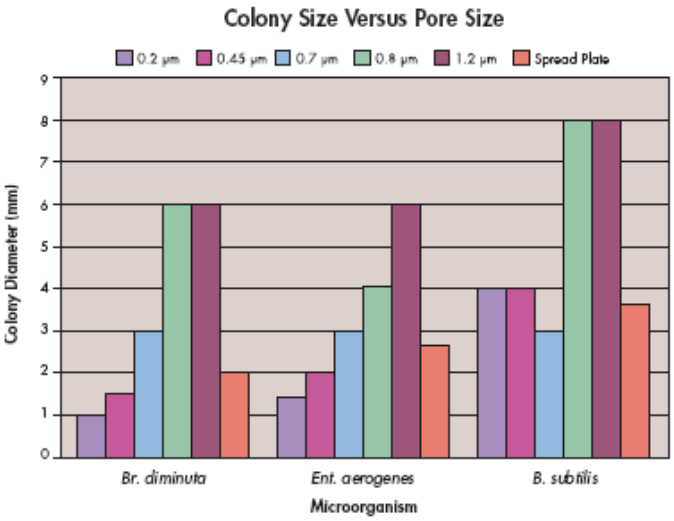
(Continued from page 3)

the test filters were tested for their retentive capabilities under the conditions of average use. Each filter was challenged with a low level of *Brevundimonas (Pseudomonas) diminuta* (the standard organism for retention testing) and the filtrate was retained for enumeration.

Results

The selection of test systems was not intended to be exhaustive but to give a broad overview of microbial recovery in relation to filter pore size. Six different filter pore sizes were tested with 12 microorganism/media combinations that are representative of the types of microorganisms encountered by those using the membrane filter (MF) technique.

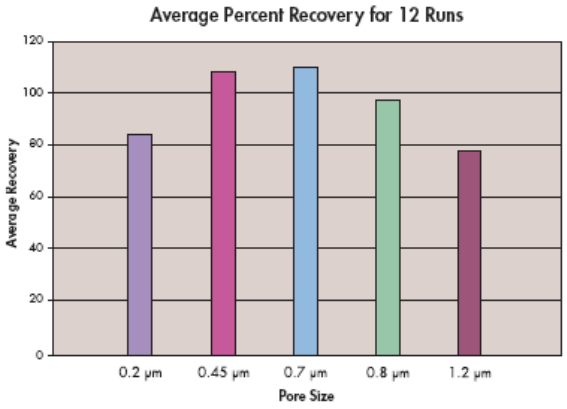
Colony Size



- Three test systems, *Br. diminuta, Ent. aerogenes, and B. subtilis,* showed differences in colony size with pore size.
  - Colonies grown on 1.2 and 0.8 µm filters were larger than colonies grown on other filter pore sizes or spread plates.
  - Colonies grown on 0.7 µm filters were the same size as, or slightly larger than, colonies grown on spread plates.
  - Colonies on 0.45 and 0.2 µm filters were the same size as, or some what smaller than, colonies grown on spread plates.
- Other test systems showed virtually no difference in colony size with any of the other pore sizes as compared to colonies grown on spread plates.

Microbial Recovery

- 0.45 µm and 0.7 µm filters demonstrated acceptable recovery (90% versus spread plates) for all 12 test systems.
- Although the average recovery for 0.8 µm filters was acceptable over the 12 test systems, the pore size had lower recoveries than 0.45 and 0.7 µm filters.
- Although 0.22 and 1.2 µm filters gave acceptable recoveries with some systems, their average recovery was significantly lower overall.



The average performance of each pore size was determined using all the test systems

Retention

- The larger pore sizes (1.2 and 0.8 µm) allowed significant passage of a small organism at low challenge levels (starved *Br. diminuta*) but there was no passage with the 0.45 or 0.22 µm pore sizes.
- Although 0.22 µm filters retained the challenge, the average recovery across all test systems was lower than 0.45 µm filters.
- Passage might be one reason why larger pore sizes (> 0.7 µm) showed lower recoveries than smaller pore sizes.

Overall

Recovery is much more complex than the retention of microorganisms on the surface of a membrane filter and the influence of pore size. It is a combination of factors that may include:

(Continued on page 8)

(Continued from page 4)

mentation of the postponed harmonized General Chapters prior to the May 2009 date is at the discretion of the user and may be subject to regulatory consideration." (1)

The three chapters (<61>, <62> and <1111>) were republished in the "Interim Revision Announcement"† section of that issue, each with the notation that the chapter was official May 1, 2009 (2-4).

"Harmonized" Bacterial Endotoxin Test

It turns out that the bacterial endotoxin test wasn't really harmonized after all. The May/June 2007 issue of *Pharmacopeial Forum* contained a revised, harmonized proposal (5). The new text has been proposed to enhance clarity and presumably make it easier for the user. The document has been re-introduced into the harmonization process as a STAGE 4 document (see discussion of <1196> below) and published in the "Harmonization" section of *PF*.☼

Water Chapters

Two of the water testing chapters had proposed revisions in the May/June 2007 issue. These both appeared in the "In-process Revision"◊ section of that issue of the *Pharmacopeial Forum*.

<643> Total Organic Carbon is proposed to be changed (6):

"This chapter has been modified in response to inquiries regarding the following topics: (1) to address the relationship between TOC and microbiological activity, (2) to provide guidance to the analyst on the method, and (3) to provide emphasis on the allowance of on-line measurements."

<645> Water Conductivity is proposed to be changed as follows (7):

"This chapter is being modified by the addition of a verification step on the entire conductivity-measuring instrument to increase the assurance of using a properly calibrated system. Individual electronic and sensor calibration steps are already in place. The addition of the verification step aims to ensure the use of a properly calibrated instrument for the measurements in low conductivity ranges and to prevent the oversight of unique electronic effects. Also being added to the chapter is a section

(Continued on page 6)

† The "Interim Revision Announcement" section is described by USP as "Standards that have been adopted and will become officially binding on the specified date."

☼ The "Harmonization" section is for the publication of "Items the Pharmacopeial Discussion Group (PDG) is working to harmonize internationally."

◊ The "In-process Revision" section is for the publication of versions of proposed revisions that are nearing completion and may be adopted soon.

(Continued from page 5)

on planned conductivity testing of monographed waters. The proposed limits in this test will be harmonized with existing conductivity tests in the current *European Pharmacopoeia.*"

<1010> Analytical Data - Interpretation and Treatment

This chapter is a really useful one, but one that is highly contentious (statisticians *may* be more argumentative than microbiologists). A proposed revision to this chapter appears in the July/Aug 2007 *Pharmacopeial Forum* as an "In-process Revision." The briefing for this chapter states:

"The Statistics Expert Committee has addressed several outstanding issues regarding this general information chapter. The following changes are proposed:

1. Add a reference pertaining to the testing of normality and a reference on sample size for equivalence studies.
2. Add an explanation on the proper use of Dixon's Test for the detection of outliers for the one-tailed case versus the two-tailed case.
3. Add a statement in Appendix B: Precision Study explaining how the variance of the mean decreases and, as a result, the precision of the experiment is improved as the number of runs and the number of replicates per run increase.
4. Change "relative standard deviation" to "percent relative standard deviation" ("RSD" to "%RSD").

In addition, typographical errors regarding the F value and the degrees of freedom in Table la have been corrected."

<1196> Pharmacopeial Harmonization

"It is proposed to update this general information chapter to include the following changes. The PDG working procedures were revised in October 2006, and thus the text of <1196> has been aligned to reflect those changes, most notably the addition of Stage 6C - *Indication of Harmonization*. Secondly, the *Status of Harmonization Tables* have been
updated to reflect the current harmonization stage for each monograph and general chapter."

For the record, the steps in harmonization (as described in this draft) are:

Stage 1: Identification
Stage 2: Investigation
Stage 3: Proposal for Expert Committee Review
Stage 4: Official Inquiry
Stage 5. Consensus
  5A. PROVISIONAL
  5B. DRAFT SIGN-OFF
Stage 6: Regional Adoption and Implementation
  6A. REGIONAL ADOPTION
  6B. IMPLEMENTATION
  6C. INDICATION OF HARMONIZATION
Stage 7: Inter-Regional Acceptance

Those monographs and chapters of interest include:

(Continued on page 7)

(Continued from page 6)

Monograph/Chapter	Lead Pharmacopeia	Stage
Sterile Water for Injection in Containers	USP	3
Bacterial Endotoxins	JP	4
Conductivity	EP	2
Microbial Limits for Non-sterile Products	EP	6
Tests for Specified Organisms	EP	6
Enumeration	EP	6
Particulate Contamination	EP	6
Sterility Tests	EP	6

<1251> Weighing on an Analytical Balance

A revision to this existing general information chapter also appeared in the "In-process Revision" section of the July/ August 2007 issue of *Pharm. Forum*.

"The General Chapters Expert Committee is proposing new text for this existing general information chapter. The new text considers the following aspects of the balance and weighing process:

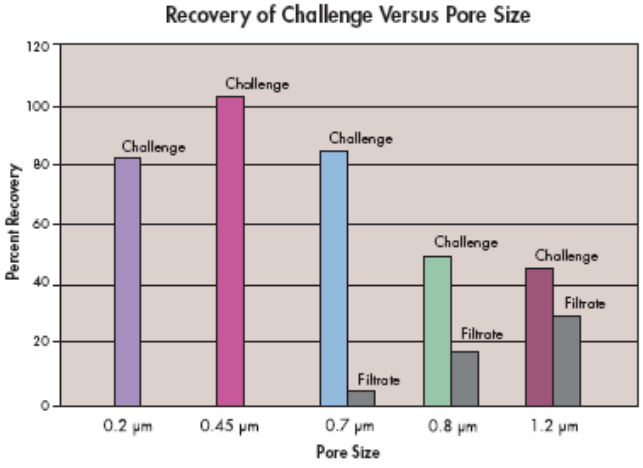
1. Installation
2. Qualification and routine checks
3. Operation of the analytical balance
4. Calibration
5. Receivers
6. Types of weighing
7. Problem samples
8. Safety considerations
9. Buoyancy corrections

The content of this new text has been aligned with the recent proposal on the general chapter Weights and Balances (41) (see page 1781 of *PF* 32(6) [Nov.-Dec. 2006]."

References

1. USP. 2007. Harmonized Microbiology General Chapters: Notice Of Postponement. *Pharm Forum*. 33 (2):168-169.
2. USP. 2007. <61> Microbiological Examination Of Nonsterile Products: Microbial Enumeration Tests. *Pharm Forum*. 33(2):189-193.
3. USP. 2007. <62> Microbiological Examination Of Nonsterile Products: Tests For Specified Microorganisms. *Pharm Forum*. 33(2):193-198.
4. USP. 2007. <1111> Microbiological Examination Of Nonsterile Products: Acceptance Criteria For Pharmaceutical Preparations And Substances For Pharmaceutical Use. *Pharm Forum*. 33(2):207-208.
5. USP. 2007. <85> Bacterial Endotoxins Test. *Pharm Forum*. 33(3):539-550.
6. USP. 2007. <643> Total Organic Carbon. *Pharm Forum*. 33(4):720-722.
7. USP. 2007. <645> Water Conductivity. *Pharm Forum*. 33(4):722-725.
8. USP. 2007. <1010> Analytical Data - Interpretation and Treatment. *Pharm Forum*. 33(4):726-736.
9. USP. 2007. <1196> Pharmacopeial Harmonization. *Pharm Forum*. 33(4):751-755.
10. USP. 2007. <1251> Weighing on an Analytical Balance. *Pharm Forum*. 33(4):756-765.

(Continued from page 4)



- The microorganism species and its condition - each microorganism has the potential to react differently
- The sieving effects of the pore size as it relates to the retention of specific microorganisms
- Type of medium and selectivity
- Structure and chemistry of the membrane filter
- Environmental conditions (e.g., moisture, incubation, temperature)

The effect of filter pore size on any specific microorganism/medium combination is not always predictable. If pore sizes other than those indicated by industry standards are used, they should be validated on relevant samples and media and compared to 0.45 µm.
This study confirmed that the standard 0.45 µm pore size is the most appropriate for general microbiological purposes. The 0.45 µm filters gave the most consistent recoveries across a variety of test systems and did not allow passage of the standard 0.2 µm sterilizing filter challenge microorganism, *B. diminuta,* under typical filtration conditions.

Conclusion

A membrane pore size larger than 0.45 µm can increase flow rate, throughput, and, occasionally, colony size (which makes the colonies easier to count). However, these larger pore sizes may not have sufficient retention for some microorganisms. Therefore, they are not well suited for total count applications.

Larger pore sizes can be used for enumerating specific organisms, such as fecal coliforms or yeast. They can also be used for difficult-to-filter samples where improved throughput or larger sample volumes are needed. In both cases, the filter's retention performance should be documented for the target microorganism(s).

Pore sizes smaller than 0.45 µm have the disadvantage of decreased flow rate, throughput, and, potentially, recovery. Therefore, the greater retentive properties of the 0.2 µm pore size have little benefit for the enumeration of bacteria, yeast, and molds in the variety of liquids considered in this study.

References

1. American Public Health Association. 1998. *Standard Methods for the Examination of Water and Wastewater,* 20th Edition. American Public Health Association. 1015 Fifteenth Street, NW, Washington, DC. Part 9000.
2. Health Industry Manufacturers Association. *Microbiological evaluation of filters for sterilizing liquids.* HIMA Document No. 3, Vol. 4, April 1982. 34 pp.
3. J. Carter, "Evaluation of Recovery Filters for Use in Bacterial Retention Testing of Sterilizing-Grade Filters." *PDA J Pharm. Sci. Technol.* 50(3):147-153 (1996).

Upcoming Events

September

17 & 18 - PMF 2007 Cosmetic Microbiology Conference
Newark, NJ
http://www.highpeaks.us/2007/cosmetic/

October

11 & 12 - 2007 PMF Fall Forum
Rochester, NY

November

5 & 6 - 2007 Bacterial Endotoxin Summit
Philadelphia, PA

Offering In-House Courses on Microbiology/ Aseptic Processing:

- The Microbiology Network/High Peaks Associates
- http://www.highpeaks.us/in-house.htm
  Experienced in custom-designed courses for the lab or the manufacturing facility, including:
  - GMP for the Microbiology Lab
  - Microbiology for Manufacturing
  - Microbiology for Management
  - Auditing the Microbiology Function
  - Investigating Microbiological Data Deviations
- USP
  Contact Steven Paul (stp@usp.org) for information on the course "Fundamentals of Microbiological Testing"

USP Corner

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

Discussion List Update

PMFList:
Number of Subscribers: 2,475
Number of Countries: 64
Number of Messages Last Month: 289

PSDGList (Pharma Stability Discussion Group):
Number of Subscribers: 988
Number of Countries: 24

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

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

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

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

Recent News

Recent News - Medical Device

Bill to Strengthen FDA Passes House
The House of Representatives overwhelmingly passed reauthorization of the Prescription Drug User Fee Act (PDUFA), enhancing the FDA's safety programs, by a vote of 403-16. The bill, H.R. 2900, will next go to a conference committee to work out differences with the Senate version (S. 1082), which was passed in May 2007.

Philippines Announces Launch of Medical Device Authority
The Philippines recently began the transfer of responsibility from the Bureau of Food and Drugs (BFAD) to the new Bureau of Health Devices and Technology (BHDT) for the registration and licensing of medical devices and device importers and wholesalers, according to documents posted on the BFAD website.

New European Medical Device Directives Expected This Fall
New European medical device directives are expected to be adopted this fall, implemented by the end of 2008 and enforced by 2010, Cristiana Spontoni, European partner with Squire, Sanders & Dempsey, said at the Fourth Annual FDAnews Medical Device Quality Congress.

Recent News - Pharmaceuticals

Production changes, layoffs planned at Pa. pharmaceutical plant
Johnson & Johnson's McNeil-PPC subsidiary plans to make production changes and begin cutting up to 340 jobs starting in September at its Lititz manufacturing and distribution facilities.

US Universities Begin Trial on Microbicide Developed by Australian ...
The University of South Florida and University of Puerto Rico have commenced a two-week clinical trial to test the microbicide VivaGel, officials with the Australian pharmaceutical company Starpharma, which is developing the product, announced on Tuesday, the *AAP/Sydney Morning Herald* reports (*AAP/Sydney Morning Herald*, 7/10). FDA in January 2006 granted an accelerated review of VivaGel.

Teva Fungus Drug Gets FDA Nod
Teva Pharmaceutical Industries Ltd. said Tuesday the Food and Drug Administration granted the generic drug developer final approval to market a generic version of Novartis AG's fungus treatment Lamisil.

Microbes have the last word. A drastic re-evaluation of antimicrobial treatment is needed to overcome the threat of antibiotic-resistant bacteria
an article by Julian Davies

JOBS

QC Microbiology Technician #CL1051
Candidate performs laboratory testing on samples (drug and devices) for the presence of contamination, submitted for analysis by using gel-clot, turbidimetric or chromogenic methods. **Headhunter posting.**

Director, QC Microbiology
Located in central NJ, this is a biopharmaceutical company dedicated to developing breakthrough biologic medicines in the area of oncology. The Company has utilized the many advances made in the fields of molecular biology, oncology, genomics, and antibody engineering to build a novel pipeline of product candidates designed to address specific genetic mechanisms involved in cancer growth and development. **Discovery Solutions LLC**

QC Micro Supervisor
Provide day-to-day oversight of the QC Microbiology drug substance testing group supporting commercial drug substance manufacturing. Allocate resources to assure cost efficient, expedient processing of samples while achieving high standards of compliance. **Wyeth**

Manager QC Microbiology
We are seeking an experienced Microbiologist to manage our QC department in our Cumberland facility. The position will involve hands on lab work as well as supervision of one direct report. Incumbent will conduct QC testing of raw materials, in-process, and finished products. Candidates should have 2-4 years' experience with aseptic technique, plating cultures, microbial IDs, and staining. Experience in a GMP environment a huge plus. Previous supervisory experience preferred. **Headhunter posting**

QC Microbiology Manager
Seeking an experienced microbiologist to manage raw material, in process, and finish product QC testing functions in a cGMP environment. **On assignment lab support.**

QC Manager Microbiological Services
This individual will be responsible for providing leadership and technical management of QC Microbiology services in Billerica. The duties include meeting final release testing and compliance objectives. Responsible for overseeing multiple laboratory areas sterility testing microbial characterizations facility monitoring microbial raw material testing and microbial support to R&D. **Bristol-Myers Squibb**

QC Analyst II - Microbiology - (FY08-112)
Responsible for routine QC Microbiology lab testing and utility/facility environmental monitoring in support of product development (injectable and pulmonary) and Commercial product. Job Duties: Samples and tests High Purity Water systems, clean steam and compressed gases for bioburden, endotoxin, conductivity and Total Organic Carbon. Performs environmental monitoring in the classified cleanroom manufacturing areas at Massachusetts facilities. **Alkermes, Inc.**