PMF NEWSLETTER

A PUBLICATION OF THE PHARMACEUTICAL MICROBIOLOGY FORUM
Distributed Internationally to 8,075 Subscribers in 87 Countries

PURPOSE: To provide a forum for discussion of microbiology issues in the pharmaceutical and related industry. The information contained in this newsletter includes the professional opinions of individuals and does not represent the policies or operations of any corporation or government agency to which they may be associated. *PMF Newsletter* is intended to serve as an open forum. The information in *PMF Newsletter* is solely for informational 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 to stimulate discussion and are not necessarily 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.

Volume 13, Number 2 February, 2007

Editor's Message	1
Cryopreservation of Bacteria - Liz Kerrigan	2
Are Microbiologists Developing R^2 Envy? - David Porter	4
The New USP Chapter "<1072> Disinfectants and Antiseptics" - Scott Sutton	7
Upcoming Events	9
Discussion List Update	9

Pharmaceutical Microbiology Forum (PMF) 2006 Editorial Board

President/Editor-in-Chief:

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

Editorial Board:

Ziva Abraham, Microrite, Inc., USA
Phil Geis, Proctor-Gamble, CTFA, USA
Klaus Haberer, Ph.D., Ph. Eur., Germany
Karen McCullough, Roche Labs, LAL User's Group, USA
Paul Priscott, Ph.D., AMS Labs, Australia
Eric Strauss, Teva Pharmaceuticals, Israel

The PMF gratefully acknowledges the support of our sponsors

Fast Times for Microbiology

There are a lot of interesting things going on at the moment. Overall, this is a good thing. We have a major push to approval of rapid microbiological methods, a major push to better understand our manufacturing processes though PAT (this of course follows FMEA, Six Sigma, TQM, and several others - all of them good ideas). The problem with this situation, though, is that we might become distracted from the basic requirements of the laboratory.

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

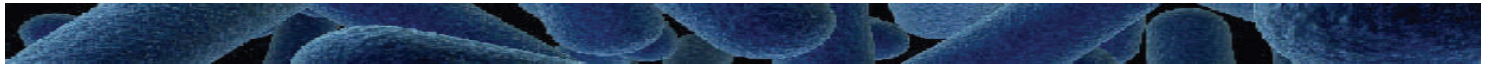
Liz Kerrigan from ATCC provides an excellent overview of long-term storage methodology for bacterial cultures. The obvious concern with storing bacteria is to maintain the viability and the purity of the culture. She describes several appropriate techniques.

David Porter provides an entertaining review of the question of the use of the correlation of determination to provide an *accurate* measure of the accuracy and precision of a microbiological method. While his article looks to its use in validating an alternate microbiological method, we are being asked ever more frequently to provide measures of uncertainty in our assays. This is a very useful discussion for us all.

Finally, USP has recently finalized several new chapters. We have focused recently on the Microbial Limits chapters, but in this issue we look at another - the new chapter on Disinfectants and Antiseptics. Reviews of other new chapters will continue in the following months.

Scott Sutton scott.sutton@microbiol.org

The *PMF Newsletter* is published by the Pharmaceutical Microbiology Forum. Copyright ©2007 by the Pharmaceutical Microbiology Forum. All Rights Reserved. Send all inquiries, letters, and comments to scott.sutton@microbiol.org.



Cryopreservation of Bacteria

Liz Kerrigan
ATCC

Cryogenic preservation is the act of freezing and storing cells at very low temperatures. Cryopreservation of living bacteria offers a way to stabilize cultures for long-term storage. Proper cryopreservation allows for the generation and maintenance of bacterial stocks and decreases the need for repeated subculturing, which can lead to contamination, genetic drift or mutation as continuously smaller portions of a population are selected. Low-temperature storage greatly reduces phenotypic and genotypic drift and helps to ensure reproducible results in a series of quality control tests using a consistent stock.

Several factors are critical to the stability and viability of a bacterial culture undergoing cryopreservation. Cell type, age, growth conditions, population size, cryoprotectant used, storage conditions and recovery methods all affect viability and stability. Before preparing bacterial cells for preservation, the culture's purity should be checked and its identity confirmed at the genus and species level. In addition, the culture's passage number should be checked to ensure it is in line with guidelines, such as those for USP. The purity and identity of bacterial cultures should be examined again when they are removed from the frozen state. A good cataloging and data record-keeping system is also important to prevent misidentification and duplication of material during the preservation process. These can be maintained as paper documents or preferably as electronic files. Software for freezer inventory, sample tracking and sample management for laboratories is available commercially.

1. Growth and preparation for cryopreservation

Bacteria being cryopreserved should be grown under optimal conditions on the recommended medium for each strain. The recommended media for ATCC Genuine Cultures™ can be found on the product sheet for each culture or at the ATCC website (http://www.atcc.org).

Among the factors influencing how well bacteria handle the cryopreservation process are whether or not the cells are grown in aerated conditions and at which point the cells are harvested. Microbial cells, particularly bacteria, grown under aerated conditions tolerate the stress of freezing better than statically grown or non-aerated cultures. T. Nei *et al.* (1) studied the performance of aerated and non-aerated cultures of *E. coli* after the freezing process. Microbial cells harvested in late log or early stationary phase are generally more resistant to the stresses of freezing than younger or older harvested cells (2).

Cells to be cryopreserved can be harvested from broth culture, agar plates or slants. When greater quantities are required, bacteria can be grown in broth culture and harvested by centrifugation. Cells to be frozen are generally suspended in fresh growth medium containing a cryoprotectant. For most bacteria, a concentration of 10^7 cells per mL is required for good recovery (3).

2. Addition of a cryoprotectant

(Continued on page 3)

BTF produces BioBall™, the world's most precise reference standard for microbiological testing, which is ideal for quantitative QC.

BioBall™ is a small water soluble freeze dried ball containing a precise number of organisms and is now available with 10 of the main USP organisms.

BTF can produce custom BioBall™ with anywhere from 5 to 10^8 cfu. In addition, BTF offers the service of taking pharmaceutical company's in-house isolates and producing BioBall™ with a precise number of the isolate.

BTF is based in Sydney Australia with an office in Pittsburgh PA and distributors in Europe and Asia.

For more information contact Marie Napoli at (412) 267 3073, email: usinfo@btfbio.com or visit www.bioball.us.

The PMF Newsletter thanks BTF Precise Microbiology for its sponsorship.

(Continued from page 2)

Bacterial cultures can be protected during the freezing process by the addition of chemical agents called cryoprotectants. Cryoprotectants such as glycerol penetrate the cells to delay intracellular freezing and protect against solute concentration effects (4). The choice of a cryoprotectant depends upon the type of cell being preserved. For freezing non-fastidious bacterial cultures, ATCC recommends a final concentration of 10% glycerol. Such a concentration can be obtained by adding 20% sterile glycerol to an equal volume of culture medium containing cells. Be sure to use reagent grade glycerol that is less than a year old, due to the possibility of oxidative by-products in the glycerol that may be toxic (5). Sterilize undiluted reagent grade glycerol by autoclaving for 15 minutes at 121°C. (Note: ATCC offers ready-to-use freeze media (catalog no. 20-2200) TSB with 10% glycerol for non-fastidious bacterial cultures.)

The time between the addition of the glycerol to the cells and the cooling process is called the equilibration period. To ensure that the cryoprotectant has enough time to penetrate the cells, the cells should be allowed to equilibrate at room temperature for a minimum of 15 minutes, but no longer than 45-60 minutes (6). The cryoprotectant (glycerol) may be toxic to cells if the equilibration time is longer than 60 minutes.

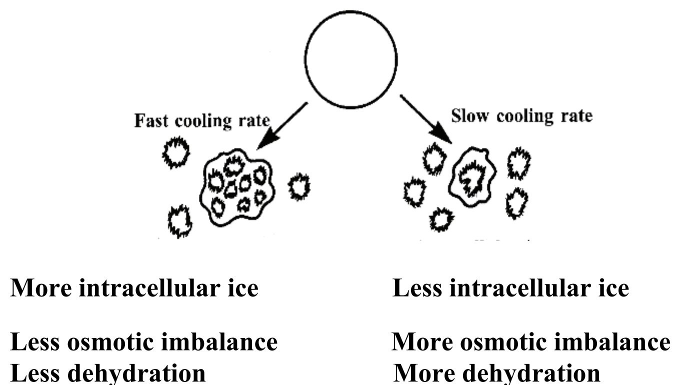
This time is usually spent dispensing the cells and cryoprotectant into the vials. The most commonly used vials are plastic cryovials (Nalge Nunc) with volumes between 1.2 ml and 2.0 ml. Be careful to freeze only one strain at a time to eliminate the possibility of mixing up vials and strains.

3. Cooling and Storage Conditions

Following application of the cryoprotectant, the cultures are ready for cooling. As cells start to freeze, ice forms first outside, then inside the cell. As the environment outside the cell starts to freeze, there is an increase in the concentration of solutes outside the cell. Because of the concentration difference across the bacterial cell membrane, water begins to leave the cell and continues to do so until a balance in solute concentration is obtained.

During this period, cooling rate is critical. Cells can become too dehydrated if cooling rates are too slow. If the cells are allowed to cool too rapidly, greater amounts of intracellular ice formation can occur. The study by Nei *et al*. on the behavior of aerated vs. non-aerated cultures concluded that the aerated cells dehydrated faster during cooling than non-aerated cells. However, some bacterial strains cannot be grown in shake culture and must be grown on agar before cryopreservation.

Representation of a Cell's Reaction to Cooling Rates (7)



More intracellular ice	**Less intracellular ice**
Less osmotic imbalance	**More osmotic imbalance**
Less dehydration	**More dehydration**

Mazur *et al*. have suggested the ideal cooling rate for bacteria is approximately -1°C per minute (8). How-

(Continued on page 6)

Internet Address	Description
http://securitytango.com/tango.php	Security Tango - A public service site dedicated to internet security with an emphasis on cleaning up viruses and spyware. Sponsored by Frontier Communications.
http://www.bacteriamuseum.org/	The Virtual Museum of Bacteria - This site brings together many links on bacteria, bacteriology, and related topics available on the web. It also provides information about many aspects of bacteria.
If you have found an Internet site that contains information of relevance to professional microbiology in the industrial sector, please let us know.	

Are Microbiologists Developing R^2 Envy?

David Porter, Ph.D.
Vectech Pharmaceutical Consultants, Inc.

We live in exciting times concerning the development of new microbiological methods. At the recent Rapid Micro User Group (RMUG) meeting held in January of this year, it was announced that Alcon had received FDA approval for the use of an alternative, rapid method for sterility testing, thus obviating the need for performing the test in chapter <71> *Sterility Tests* in *USP* 30. I congratulate all the members of the extended Alcon team for their hard work and perseverance. I would also like to congratulate the FDA for standing by their word. They asked for such alternative method proposals, they stated they would review them with an open mind, and they have fulfilled their promise.

In retrospect, it is perhaps not surprising that that sterility test could be replaceable by an alternative method. Yes, indeed, in retrospect one can predict with a high degree of accuracy that the Chicago Bears lost to the Indianapolis Colts in this year's Super Bowl! Nonetheless, consider that the sterility test has a binary outcome. An article either is or is not sterile. No number of viable microorganisms can exist in a tested article that is truly sterile. In that regard, there is no variance allowed. That makes the sterility test rather special among the various types of microbiological testing that is performed. Clearly an article under test required to meet a pharmacopeial limit for total aerobic microbial count can have a range of cfu found/g and still pass the test. An article under test for the absence of a specified microorganisms, say. *E. coli*, can in fact have a positive turn up in the test, but be cleared, so to speak, by a more specific identification test. Clearly variance of results within a tested article is allowable and still permit passage.

Many of the newer microbiological test methods are presented with claims for having very high precision and accuracy. Often results from these new methods are presented with linear regressions having very high r^2 (coefficient of determination) values. A high r^2 value indicates that a high percentage of the variance in the data may be predicted from the parameter being measured. For example, if the cleavage of a nonfluorescent ester by intracellular esterases is highly correlated with the presence of a viable microorganism, this would translate to a high correlation coefficient r which naturally translates into a high r^2. Such results are a hallmark of a modern analytical chemistry method.

Reducing variance is a good thing, right? I suggest that the answer to this is yes and no. If you have an article under test for sterility, you would like to get the vari-

(Continued on page 5)

MicroBioLogics®

MicroBioLogics, Inc. is a leading producer of lyophilized microorganisms for quality control in microbiology laboratories worldwide. Specializing in clinical, industrial, food quality, environmental and educational markets, there are over 3,000 items in the product line with more than 500 different lyophilized microorganism strains. MicroBioLogics, Inc. is a globally known and rapidly growing company based in St. Cloud, Minnesota USA with 35 years of experience. The people behind MicroBioLogics attribute their success to their enduring commitments to product quality, business integrity and customer satisfaction. The business of supplying reference stock culture preparations means commitment to the customer throughout the process: from the selection of strains, throughout the preparation and lyophilization processes, to comprehensive after sales support. This winning strategy provides MicroBio-Logics with strong customer loyalty and the customer with a superior product.

The products manufactured by MicroBioLogics are easy to use, save your laboratory valuable time and money, and are of guaranteed quality. The MicroBioLogics product line supports four basic areas of quality assurance: laboratory performance, procedure performance, product performance and personnel performance. Reference stock cultures, enumerated reference stock cultures and quality control slides are the three main groups that make up the MicroBioLogics product line. FDA registration, ISO: 9001:2000 certification, CE Mark product conformity and ATCC Licensed Derivative™ products are evidence of a manufacturer standing behind its product quality. Because quality matters most, it is important that your lab chooses trusted and dependable products to meet quality control challenges.

The PMF Newsletter thanks MicroBiologics for its sponsorship.

(Continued from page 4)

ance in results not accounted for by your measured parameter as close to 0 as possible (r^2 approaching 1). Again, for a sterility test, this is extremely important, as any unexplained variance could result in the potential release of a contaminated lot. The reality of a sterile article is that it must have no viable microorganisms in it. The sterility test in chapter <71> is intended to address this requirement for an article, and we can be certain that the recently approved method will have demonstrated that it produces results at least as good as the compendial method. That is to say, it will have no more unexplained variance than the compendial method. However, the reality for the entire lot of product may not be addressed by the testing of a relatively few samples. This weakness of the current chapter <71> method is well-established. Unless the newly approved method deals with this weakness (and it might—an alternative method can certainly be superior to the compendial method) the same risk still exists. No matter how high the r^2 value may get with such an approach, the reality of the entire lot may not be reflected by the result, and I suggest that we not lose sight of the forest for the tree.

This sort of question reminds me of one of the better ways to express the concept of an emergent property. Imagine that you are given a tissue culture dish with a single neuron in it. Further imagine that you are allowed to learn everything this is to learn about this neuron. Do you believe your level of knowledge would rise to the degree where you could imagine a collection of these achieving consciousness? A good microbiological method? I would guess not.

As scientists, we are always striving to improve our abilities to discern what is real. Biology is a complex field. By this I don't mean that it is difficult to master (it is), but rather that it is characterized by multiply interactive nonlinear behaviors leading to large scale unpredictability. In other words, biological reality is filled with variance, and variance that indeed reflect reality. Therein lies the crux of this article and thus its title.

Would you like to sponsor the *PMF Newsletter*?

Opportunities to sponsor the operation of the *PMF Newsletter* are available.
Please contact the editor for details.

Analytical chemistry methods are typically ones with low variance and therefore, high r^2 values. For many of the articles tested by analytical chemistry methods, this is probably close to reality. It is expected that at the level we are interested in, a solution is indeed homogeneous and therefore a small sample taken from a large volume of solution should reflect the reality for the whole volume. The reality of such a solution is indeed one with low variance and thus high r^2, of course depending upon the parameter measured. If that same article is exposed to conditions that bring about turbulence, that condition is indeed complex.

By fixating on developing microbiological methods with high r^2 values, thus ones with little variance not accounted for by the parameter being measured, is it possible that microbiologists are losing their feel for what is real in their test systems for the sake of methods whose results more closely resemble analytical chemistry? Again, the reality of biological systems is complex, and attempting to predict the behavior of such systems by measuring a single parameter to an excruciating level of resolution may lead to delusion. Statistics may certainly be used to derive standard curves with very high r^2 values, but statistics may also be used to handle many predictors and variates at once. The equations are messier, the results harder to explain, but at the end of the day, the results are much more likely to reflect biological reality.

I wonder whether some of this drive to analytical chemistry precision has resulted from perhaps excessive concern with compendial requirements. We know that alternative methods are to demonstrate at least equivalence with the compendial ones. However, remember that compendial methods are based on the idea of the singlet determination (see *General Notices and Requirements*, *USP* 30). They are not intended to be release tests. It is necessary for an article to comply with the specification in USP should a monograph exist for such an article. But passage of

(Continued on page 11)

(Continued from page 3)

ever, most non-fastidious bacteria will withstand less-than-ideal cooling rates and can be frozen by placing the vials on the bottom of a mechanical freezer at –60°to –80°C for one hour. For more fastidious strains, more uniform cooling rates are required. A programmable-rate freezing apparatus (such as a Cryomed from ThermoFisher Scientific or a "Mr. Frosty" from NALGENE; Cat. No. 5100-0001) can be used to obtain rates of -1°C/minute.

After cooling, the vials are stored in the vapor phase of a liquid nitrogen freezer at –170°C. Storage by immersion in liquid nitrogen is not advised. Storage temperatures below –130°C are critical to the long term stability of bacterial cultures, because that is the point at which ice crystal formation stops. At temperatures above –130°C, ice crystals continue to grow and reform (9,10). Warming and recooling, even without complete thawing, can be detrimental to bacterial cultures. It is imperative that storage temperatures be maintained continuously and that the location, date and details of the freeze be recorded when the freezing process is completed. Although not practiced at ATCC, –80°C is sufficient for short-term storage for most non-fastidious bacteria.

4. Recovery

To revive frozen cultures, thaw in a 37°C water bath and transfer all vial contents to the optimum growth medium for incubation at the proper temperature. More fastidious cells may require more time to recover from freezing. Cultures should be re-checked for viability and purity after the process to ensure that the material recovered from the preservation process remains unchanged.

References

1. Nei, T., T.Araki and T. Matsusaka. 1969. Freezing and injury to aerated and non-aerated cultures of *Escherichia coli*. In T. Nei, Ed. *Freezing and Drying of Microorganisms.* University of Tokyo Press, Tokyo, Japan.
2. Heckly, R.J. 1978. Preservation of microorganisms. *Advances in Applied Microbiology 24:* 1-53.
3. ATCC Preservation Methods: Freezing and Freeze Drying. Ed. F.P. Simione and E.M. Brown, American Type Culture Collection, Rockville, Maryland, 1991.
4. Farrant, J. 1980. General observations on cell preservation. In: M.J. Ashwood-Smith and J. Farrant, Eds. *Low temperature Preservation in Medicine and Biology*, Pitman Medical Limited, Kent, England, p. 1-18.
5. Shannon, J. E., and M. L. Macy. 1973. Freezing, storage and recovery of cell stocks. In *Tissue culture, methods and applications*, edited by P.F. Kruse, Jr., and M.K. Patterson, Jr., 712-718. New York: Academic Press.
6. Cyropreservation Technical Manual, F.P. Simione, Nalge Nunc International, Rochester, New York, 2006.
7. Cyropreservation Technical Manual, F.P. Simione, Nalge Nunc International, Rochester, New York, 2006.
8. Mazur, P., Leibo, S. and Chu, E. 1972. "A two-factor hypothesis of freezing injury," *Experimental Cell Research*, **71**, 345.
9. Meryman, H. 1966. "Review of biological freezing," *Cryobiology*, Academic Press, New York, NY.
10. Mazur, P. 1966. "Physical and chemical basis of injury in single-celled micro-organisms subjected to freezing and thawing," *Cryobiology*, Academic Press, New York, 214.

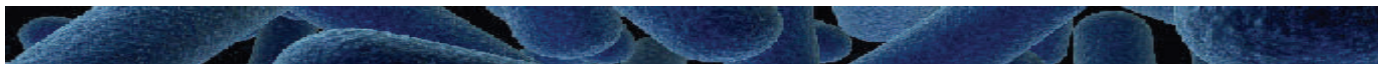
MIDI Labs, Inc. is an FDA-registered, cGMP-compliant service laboratory that provides rapid, accurate microbial identification services at competitive prices. We provide bacterial, fungal, and yeast identifications, and can identify over 2,500 species.

Our laboratory offers 16S and 28S rRNA gene sequencing using the MicroSeq® Microbial Identification System and fatty acid methyl ester (FAME) analysis using the MIDI Sherlock® Microbial Identification System. As co-developer of MicroSeq's microbial libraries and sister company of MIDI, Inc. (manufacturer of the Sherlock system), MIDI Labs has more experience and expertise using these technologies than any other service laboratory. We also provide a combined polyphasic report (DNA and fatty acid results in a single report) for your most critical unknowns.

We offer volume discounts, rapid turnaround time and long-term confidential data management. Reports are summarized and customized to meet your facility's needs.

MIDI Labs is the premier service lab for your microbial identification needs. For more information, please contact us at 302-737-4297 or info@midilabs.com.

The PMF Newsletter thanks MIDI Labs for its sponsorship.



The New USP Chapter “<1072> Disinfectants and Antiseptics”

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

USP has published 7 new chapters in the second supplement of USP 2006. We have discussed three of them in past newsletters on the topic of the microbial limits tests. This issue of the newsletter will review the new chapter <1072>.

The scope and importance of the chapter is presented in the introductory text:

> “A sound cleaning and sanitization program is needed for controlled environments used in the manufacture of Pharmacopeial articles to prevent the microbial contamination of these articles. . . The cleaning and sanitization program should achieve specified cleanliness standards, control microbial contamination of products, and be designed to prevent the chemical contamination of pharmaceutical ingredients, product-contact surfaces and/or equipment, packaging materials, and ultimately the drug products. . . In addition to disinfectants, antiseptics are used to decontaminate human skin and exposed tissue and may be used by personnel prior to entering the manufacturing area. . . This general information chapter will discuss the selection of suitable chemical disinfectants and antiseptics; the demonstration of their bactericidal, fungicidal, and sporicidal efficacy; the application of disinfectants in the sterile pharmaceutical manufacturing area; and regulation and safety considerations.”

Would you like to sponsor the *PMF Newsletter*?

Opportunities to sponsor the operation of the *PMF Newsletter* are available.

Please contact the editor for details.

The introduction notes that this chapter cannot cover all aspects of disinfectants and antiseptics, and references three common texts for further information (Ascenzi 1995, Block 2001, Russell et al 1999).

The introduction is followed by a section on definitions of common terms. This is a particularly useful section as many in the industry use the terms antiseptic, disinfectant and sterilant in imprecise ways. This section is followed by a short section on the relative resistance of different types of microorganisms to chemical agents.

Major disinfectant classes are listed next, with some generic examples. This section was much larger in earlier versions of the chapter, but in the final version this section has been reduced to a single table of different classes of disinfectants, and some text describing the factors affecting the activity of disinfectants.

A new and useful discussion in this chapter is in the next section which describes the selection of an antiseptic for hand and surgical site disinfection. Handwashing is a topic that is important to the aseptic manufacturing community, but one that does not receive a great deal of attention. This section at least underscores the need for attention to this arena. The next section discusses factors of importance in the selection of a disinfectant for a manufacturing facility.

Chapter <1072> includes a section on the theoretical discussion of disinfectant activity. This begins with a discussion of kill rate in the presence of disinfectants. It goes on to describe the concentration exponent. The casual reader might become confused in that the topics are not clearly separated (except by the initiation of a new paragraph), but the concentration exponent does not have anything to due with kill rate as such. It is, rather, a way to compare the time required for complete kill in different concentra-

(Continued on page 8)

(Continued from page 7)

tions of the same antimicrobial chemical in the same mileu (Cowles, 1939-40). The topic of the concentration exponent is also addressed (in lesser detail) in USP chapter <1227>, and it might be useful for USP to eliminate this redundancy in the near future. The most efficient means to this would be to remove it from <1227> in favor of consolidating all information on kill measurements for biocides in this one chapter

The chapter continues with a description of the commonly accepted sites of disinfectant activity. This section underscores the widespread effects of toxic chemical agents on the microbial cell. This leads into a discussion of resistant to disinfectants. The USP chapter is not supportive of the idea that microbial resistance to chemical agents is a significant issue (Sutton, 2005).

One source of some confusion in the pharmaceutical industry is the choice of appropriate methods for disinfectant efficacy testing. This chapter provides general guidelines on three methods that might be used to qualify a disinfectant for use in the manufacturing facility. The first two are laboratory tests, the use-dilution and surface challenge tests. The third test to qualify the disinfectant is particularly relevant. The USP recommendation is to perform

> "... a statistical comparison of the frequency of isolation and numbers of microorganisms isolated prior to and after the implementation of a new disinfectant. This is considered necessary because critical process steps like disinfection of aseptic processing areas, as required by GMP regulations, need to be validated, and the EPA registration requirements do not address how disinfectants are used in the pharmaceutical, biotechnology, and medical device industries."

Guidance is provided on how to conduct the surface challenge test, although not on the use-dilution nor the statistical methods to compare environmental monitoring data.

This chapter correctly stresses the need to neutralize residual disinfectant in laboratory assays. However, it provides redundant information to that provided in USP chapter <1227> provided. It is not clear why the information on neutralization is provided again, especially as the chapter cross-references <1227>. This might be another opportunity to consolidate the text in USP, placing all "informational" guidance on method suitability studies in the appropriate chapter (in this case chapter <1227>).

The final section of the new USP chapter <1072> is a summation of the role of disinfectants in a cleaning and sanitization program. There is discussion of critical program elements, the legal (21 CFR Part 211) requirements, and personnel training expectations. This summary includes a second discussion of the question of disinfectant rotation, in this instance more complete with the explicit recommendation that regular use of a disin-

(Continued on page 10)

It has come to our attention that an error has appeared in Remel's notice, claiming an SAL of 11^{-6} or 12^{-6} for their sterile contact plates. This error is the fault of the PMF, and was not in the text provided by Remel for publication. The PMF apologizes for this mistake.

remel

Remel is a customer-focused manufacturer and distributor of the highest quality microbiology products. Our complete line, routinely available from local distribution centers across the US, includes products used for sample collection through organism identification. Remel features Quanti-Cult® QC Organisms, Prepared and Dehydrated Culture Media (including both animal based and animal free media), and products specifically designed for use in Media Fill Trials. Our Sterile Contact Plates have a sterility assurance level of 10^{-6} to provide the highest quality product available, which reduces the risk of false positive contamination results. For additional information, please visit www.remel.com/products/industry or call 800-255-6730.

The PMF Newsletter thanks Remel for its sponsorship.

Upcoming Events

February

- 7th - 9th **EM Database and Trending**
  Location: PDA-TRI
  WebSite: www.pdatraining.org
- 19th - 21st **PMF Open Conference on Compendial Issues**
  Location: Baltimore, MD, USA
  WebSite: www.highpeaks.us

March

- 19th - 23rd **PDA Annual Meeting**
  Location: Las Vegas, NV, USA
  WebSite: www.pda.org

April

- April 1st - 4th **Annual Conference of the Association for General and Applied Microbiology (VAAM 2007)**
  Location: Osnabrück, Germany
  WebSite: www.conventus.de/vaam2007

May

- May 22nd—25th **Annual ASM Meeting**
  Location: Toronto, Canada
  WebSite: www.asm.org

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.

Click Here for high-impact Awareness gifts

A great source for microbiology-related ties, scarves and hats

Vectech Pharmaceutical Consultants, Inc. offers an experienced team of consultants, engineers, architects, and scientists serving the pharmaceutical, biotechnology, and medical device industries. We provide comprehensive consulting and pride ourselves on bringing a regulatory, engineering, and scientific perspective to every project - from conceptual design to finished product. We provide solutions, not just services.

Contact us at: (800) 966-8832 or email us at info@vectech.com.

Discussion List Update

PMFList:
Number of Subscribers: 2,143
Number of Countries: 64
Number of Messages Last Month: 261

PSDGList (Pharma Stability Discussion Group):
Number of Subscribers: 898
Number of Countries: 23

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

(Continued from page 8)

fectant be augmented with regular use of a sporicide. Finally, the chapter ends with a discussion of laboratory safety concerns with the use of the toxic chemicals in the lab.

This chapter fills a real need in pharmaceutical microbiology in that it directly addresses a previously ignored area. This chapter also illustrates a trend for the USP. Many requests come in to the Microbiology and Sterility Assurance Committee of Experts (USP MSA) for guidance. The desire frequently expressed is for USP to provide guidance in an area of ambiguity, frequently one in which the correspondent has recently been discussing with a regulatory representative. Each of these requests must be balanced against the resistance of USP to become a "textbook" for laboratory procedures. This resistance is reinforced by the feedback of many (other) correspondents who clearly feel that USP has gone quite far enough already in describing laboratory and QC practices. The USP is clearly moving to fill a perceived need in the pharmaceutical industry, but this effort is also being tempered by the desire to avoid compromising practice in a well-run laboratory.

This process requires feedback from the industry – write to USP. The staff liaison for the USP MSA is Radha Timuralai at rst@usp.org. Please write to him with any comments or questions. These comments should be supported by data or sound literature support.

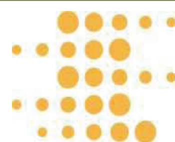
References

1. Ascenzi, J.M., Ed. *Handbook* of *Disinfectants and Antiseptics, 5th* ed.; Marcel Dekker: New York, 1995.
2. Block, S.S., Ed. *Disinfection, Sterilization, and Preservation, 5th* ed.; Lippincott Williains & Wilkins Publishers: Philadelphia, 2001.
3. Cowles, Philip B. 1939-40. The Disinfection Concentration Exponent. *Yale J Biol Med.* 12:697-704.
4. Russell, A.D.; Hugo, W.B.; Ayliffe, G.A.J., Eds. *Principles and Practices of Disinfection, Preservation and Sterilization, 3rd* ed.; Blackwell Science Inc.: London, 1999.
5. Sutton, SVW. 2005. Disinfectant Rotation - A Microbiologist's View. *Controlled Environments.* 8

RMUG 2007

The recent RMUG meeting was a great success. This meeting is unique among the plethora of "Rapid Microbiology" meetings in that it actually provides new information each year, keeping pace with advances in the field.

The major news out of this meeting was approval of a 4-hour sterility test for product release in the US. In addition, delegates had the opportunity to interact with representatives from FDA, USP, and thought leaders.



sartorius

Sartorius is an internationally leading laboratory and process technology supplier covering the segments of biotechnology and mechatronics. Sartorius has over 75 years experience in the manufacturing of cellulose nitrate membranes which are routinely used today for microbiological analysis.

The detection of microbial contamination in sample liquids such as final product, incoming inspection or during in-process testing plays a significant role in the quality assurance process. The requirements for a practical microbiological test method are that it permits quantitative and reproducible detection of trace contamination and that it can be performed efficiently and economically under routine conditions. These requirements are fulfilled optimally by the membrane filtration method. The membrane filter method is worldwide accepted and the preferred method for analyzing aqueous solutions for microbial contamination.

Sartorius offers an extensive line of high quality and reliable membrane-based solutions for all your microbial analysis needs, specifically for **microbial enumeration**, **sterility testing** and **air monitoring**. To learn more about Sartorius Microbiology Products, please visit us at: www.Sartorius.com/microbio.

The PMF Newsletter thanks Sartorius for its sponsorship.

(Continued from page 5)
the tests by the article is not sufficient to prove that the lot from which the article was derived is sufficient for release.

By concentrating so much effort on developing methods with high r^2 values when applied to small sample sizes such as required by compendial methods, are we perhaps losing sight of what is more important, namely determining if an entire lot of product is safe for release? Perhaps we are reaching a point where the test methods have more than adequate high r^2 values. Perhaps we need to redouble efforts to develop means of testing much larger sample sizes. In fact, perhaps what we need to do is find ways to marry the improved laboratory-based microbiological methods with the concepts of process analytical technology (PAT). By doing so, we reduce methodological variance (in itself a laudable goal), and improve our understanding of the real variance within the entire lot. This is admittedly a difficult challenge, but I do have to wonder if it is the next frontier for us to conquer. It would take us out of the realm of concentration on the compendial requirements and into the realm of real biological understanding, a state of affairs most of us would be happy with.

About the Author
David A. Porter, Ph.D., is a Pharma Consultant with Vectech Pharmaceutical Consultants, Inc. In that role, he serves individuals/companies that may have general needs pertaining to microbiology, toxicology, biostatistics, managerial processes, and training material development.

Previously, he was Director of the General Chapters Group, Drug Standards Development at the United States Pharmacopeia. In that role, he was responsible for most of the general chapters in the USP. He arrived at USP in February of 2000, where he was involved in a broad range of biological aspects pertaining to both monographs and general chapters while in the Complex Actives division.

Previous to USP, Dr. Porter worked for pharmaceutical and cosmetic companies in the areas of biology and statistics.

Dr. Porter can be reached at dporter@vectech.com.

BUSINESS DEVELOPMENT MANAGER
RARE PHARMACEUTICAL MICROBIOLOGY
SALES OPPORTUNITY!

Location flexible but preferably NY, NJ or PA
Need to travel extensively within the US
Initial training in Australia

Do you thrive on success?

BTF Precise Microbiology is a rapidly growing Biotech company, based in Sydney Australia, supplying the world's most precise quantitative biological reference materials and standards to Life Sciences worldwide.

As an established market leader in US water testing, we now wish to rapidly grow our business within the US pharmaceutical industry. BTF is seeking the services of an experienced Pharmaceutical Business Development Manager to join their dynamic Sales & Marketing team.

The Ideal Candidate:
- Degree in one of the Life Sciences - preferably Microbiology
- 5+ years selling laboratory consumables or laboratory equipment to the US pharmaceutical industry
- Proven success in developing new business with pharmaceutical laboratories
- Ability to work independently
- Results driven

If you aspire to work with a market leading Biotech Company selling the highest quality products, send your resume to – chris.howard@btfbio.com

BTF website - www.btfbio.com

High Peaks Associates offers conference planning services to the pharmaceutical, medical device and personal products industries. Go to http://www.highpeaks.us/conference.html to see how you can easily put on your next conference, no matter the size.

High Peaks is now a **Certified CEU Provider** by IACET. Contact us to **provide CEU at your seminar or conference.**