PMF NEWSLETTER

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An Updated Vitek and a Clean Lab

The Vitek 2 Compact has been at all the trade shows for over a year now. In this issue of the newsletter we hear from a user of the equipment who provides his evaluation of the system. It should also be mentioned that bioMerieux is now bundling the Vitek 2 Compact and the Bacterial Barcodes system (acquired by bioMerieux recently) to provide both a phenotypic and a genotypic system for microbial identifications.

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

We continue to review the new USP chapters in the newsletter. This month we take a look at the chapter <1117> Best Microbiological Laboratory Practices. Guiding principles to this might be conveyed as clean everything, separate and control, and check all incoming materials to the best of your abilities. This seems commonsense now, but when the chapter was first released to *Pharmacopeial Forum* several of the recommendations were viewed as overly cautious. This review provides some perspective for the chapter.

Finally, we had a very successful conference this month on GMP in microbiology. Our thanks to all the participants and speakers. Next month we have a great looking conference on validation concerns that treats "method suitability studies" and "validation of alternate methods" as two points on the "validation" continuum. Come join us in Texas!

2007 PMF Conference on
Validation in Microbiology
May 14-15, 2007
http://www.highpeaks.us/2007/val

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Vitek 2 Compact: Its Use in a QC Microbiology Lab

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The Vitek 2 Compact (V2C) is the latest microbial identification system made by bioMérieux, Inc. It is a smaller version of Vitek 2 and like it, it uses phenotypic technology to achieve its results like its predecessors, Vitek Legacy and API strips. However, it is far more advanced and streamlined. The V2C has reduced the amount of time needed to set up the identification and the time needed to complete it, all while increasing the accuracy of the results via an enhance database. It is easy to use and is 21 CFR part 11 compliant, using technologies such as electronic signatures, a comprehensive audit trail and an electronic review/approval process.

Operation Overview

Most microbiologists working in the pharmaceutical industry have at some point worked with, or at least encountered, the Vitek Legacy (aka the original Vitek), Vitek 2 or V2C. For those that haven't, I will give a brief rundown on the workings of the V2C with some comparisons to the Vitek Legacy. Since in our lab we only use the V2C and have used the Vitek Legacy in the past, in this article I will not speak to the abilities of the Vitek 2.

The overall sequence of events is similar when using the Legacy version or the V2C. With both systems, the user will first create a cell suspension of a designated density. The density is measured with a DensiChek for the V2C. This measuring instrument is provided by bioMérieux. The Vitek Legacy uses a similar instrument, called a colorimeter. After achieving desired cell concentration, the suspension is loaded into the ID card. The card itself has an array of wells filled with different substrates (e.g., carbon sources). There are different cards for different types of microorganisms. For example, the GN card identifies Gram-negative rods and the BCL card identifies Gram-positive rods with spores. The microorganism type is determined by a gram stain. The previously prepared suspension is then drawn up into the card through a straw that is attached to the card. If using the Legacy, the straw has to be manually inserted. The drawing of the suspension into the card is done by placing a tray of cards into a chamber, which first creates a vacuum and then forces the air back into the compartment, making the suspension move up the straw and into the wells of the card. Following this, the tray with the card(s) is moved into a second chamber, which cuts off the straw and seals the card, reads the barcode and places the cards into the incubator. Cards are removed from the incubator every 15 minutes, interrogated by transmittance optics, and then returned to the incubator. In the Legacy system, the cutting of the straw, the sealing of the cards, and the placing of the sealed cards into the incubator is done manually.

Just up to this point, it is clear that the V2C has saved valuable set up time. In addition, time is also saved on

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not requiring any demarcations to be made on the cards. All sample information is entered directly into the software and the cards are scanned manually, using a barcode scanner, to assign each card a sample number. The barcode on each card has the lot number, expiration date and an identifier number that is unique to each card. A nice feature that was added to the software is that it prevents the user from utilizing an expired card. This helps enhance compliance without any extra work.

As I mentioned earlier, the V2C reads each card every 15 minutes. The system reads the colorimetric and turbidity changes within the wells of the cards and determines whether the reaction did or did not occur. The results are compared to a database of known organisms and a final identification is generated by the system. Some of the more reactive organisms like *E. coli* and *S. maltophilia* can ID in as fast as 2 to 4 hours. Some of the tougher bugs to ID can take up to 18 hours. These include yeasts (18 hours) and Gram-positive rods with spores (14 hours). Most organisms are finished within 8 hours. This allows the process to be completed within one day, which can be a great advantage with reporting results and completing investigations.

By eliminating a number of steps, this system has made performing microbial identifications a more routine task. The ease of use of the V2C has also decreased the time needed to train personnel. Once the user is comfortable with performing a Gram stain, he/she can easily be trained on using the V2C. Reduced training time is a valuable commod-

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The 2007 PMF GMP Conference

The 2007 GMP conference just completed in Philadelphia. This year the format changed slightly to more of a workshop and discussion format than a strict lecture style. This change was greeted with enthusiasm from the participant,s who has the opportunity to interact with FDA (retired) lecturer Ed Firzgerald (of Fitzgerald Associates), as other thought leaders.

This year's conference had heavy representation from non-sterile manufacturers, and much of the discussion involved issues peculiar to this area. Given the attention vaccine manufacturers has recently received, it was no surprise that this topic also become one that was extensively evaluated from the perspective of GMP procedures in manufacturing and the microbiology laboratory.

We were fortunate to have Don Singer among the speakers. He is a Director at the American Society for Quality and the chairman of the ASM's committee on industrial microbiology for the National Registry of Microbiologists. Dr. Singer provided a strong discussion of Quality tools available to the microbiology lab, and lead an engrossing practical on the use of FMEA in microbiology problem-solving.

This meeting received strong endorsement from the attendees and will be repeated next year. It was also remarkable in that it is the first meeting for which PMF has been able to offer Continuing Education Units (CEU) through the organizing company High Peaks Associates (http://www.highpeaks.us).

Internet Address	Description
http://www.doctorfungus.org/	Doctor Fungus is an extensive on-line reference to all things mycological
http://dmoz.org/Science/Biology/Microbiology/	The Open Directory Project is the largest, most comprehensive human-edited directory of the Web. It is constructed and maintained by a vast, global community of volunteer editors.
If you have found an Internet site that contains information of relevance to professional microbiology in the industrial sector, please let us know.	

USP <1117> Microbiological Best Laboratory Practices

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Introduction

The USP finalized a series of chapters this summer. Past issues of the newsletter have discussed four of these:
<61> PMF Newsletter vol 12 no3 (March, 2006)
<62> PMF Newsletter vol 12 no4 (April, 2006)
<1111> PMF Newsletter vol 12 no6 (June, 2006)
<1072> PMF Newsletter vol 13 no2 (Feb, 2007)

In this issue we will examine the recently released chapter "<1117> Microbiological Best Laboratory Practices" This chapter came about after a great deal of soul searching on the part of the microbiology committee over the conflicting expectations on the part of industry of the role of USP chapters and the practice of pharmaceutical microbiology. On the one hand are the established, large companies with well-entrenched microbiological practices. Their earnest desire, as a rule, is for USP and all other regulatory agencies to remain constant and not meddle in business decisions. This is completely understandable. On the other hand, there are the small companies who might be a bit uncertain about how to adequately support the microbiological quality of their products. These companies have repeatedly requested more information as to how microbiology should be conducted in the pharmaceutical environment.

There is a need to set some base level of microbiological laboratory standards. Therefore, after several rounds of discussion (stretching over a couple years), the AMB has presented a draft chapter entitled "<1117> Good Microbiological Laboratory Practices" (1). The response to this initial proposal was spirited. Comments can in from industry and FDA on this initial proposal, and a draft revision was published in 2004 (2). This revision was well-received (few comments) and made effective (with minor changes) in August of 2006 (3).

Chapter Organization

The chapter is meant to provide minimum guidance for microbiological practices in the pharmaceutical environment. As such, it is organized into topics of importance to the microbiologist:

- Media Preparation and Quality Control
- Maintenance of Microbiological Cultures
- Maintenance of Laboratory Equipment
- Laboratory Layout and Operations
- Training of Personnel
- Documentation

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- Maintenance of Laboratory Records
- Interpretation of Assay Results

Media Preparation and Quality Control

The quality of work in a microbiological laboratory depends on the quality of the culture media. It is essential to use the correct media for the purpose at hand, although the correct media is not always obvious. For example, water testing is commonly done with R2A agar, but many facilities use TSA for this purpose. The recommendation is provided that the choice of media should be consistent, appropriate and justified.

The proposed chapter spends some time on media preparation as well. The recommendations include accurate weighing of dehydrated components, the use of high quality (USP Purified) water, completely dissolving the dehydrated media or individual ingredients, and the need to control the heating of the media to avoid damaging heat-labile components of the media. Some recommendations on the labeling and packaging of media are also provided.

An entire section is devoted to the question of media storage and the effects this might have on the media quality. Excesses of heat and cold are to be guarded against, as is the potential for dehydration of poured plates. Some guidance is also provided in quality control for molten media used in pour plates.

The quality control of the media is a critical concern. Interestingly, initially some of the most passionate commentary on the chapter dealt with the "excessive" amount of space provided to media quality checks. Since the initial release in 2003, however, the harmonized Sterility Tests and the harmonized Microbial Limits Tests have both incorporated stringent media quality checks.

Maintenance of Microbiological Cultures

Second only to media, safeguarding the stock cultures is the most important component of a successful microbiology laboratory. These must be handled carefully at all times to avoid contamination.

The care of the cultures starts upon receipt. A careful stock culture curator will confirm the identity of the received cultures, even if they come from as respected a source as a national culture collection. Mistakes can happen. The use of an incorrect strain in a compendial test could bring the results of weeks or months of work into question.

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ity in any industry, but especially in Pharma where so much attention is paid to proper training methods.

21 CFR Part 11 Enhanced Software

The V2C also has compliance advantages. This system was designed to satisfy 21 CFR Part 11 requirements. To start, it has an excellent audit trail system. First, each isolate has its own audit trail, which includes details of the identification process (times, dates, etc.) and any changes and notes that pertain to that isolate. Second, there is a system audit trail, which includes system failures, back up times, archival times and error messages. There is also an audit trail that details the security information of the system, such as successful and unsuccessful log in attempts, log out attempts and information on locked out accounts. Because all these events are recorded, the need to keep a logbook for tracing them is eliminated. Furthermore, the V2C has a system of reviewing and approving the isolate reports. This function is customizable and can be set up to review alone, review and approve or neither. Our procedure has been for the analyst performing the identification to review the results and for a supervisor or designee to approve them. All reviews and approvals (and logging in for that matter) are handled through electronic signatures. The system prevents the reviewer from approving the same isolate and only the logged in user can perform either action.

There are three user levels in the V2C. They are administrator, supervisor and user. The administrator has all rights in Windows XP, but no rights in the V2C software. The supervisor has all rights in the V2C software, but nothing more than logging in and out in Windows XP. Lastly, the user has limited rights in both the V2C software and the operating system, which essentially allow him/her to perform identifications and review their work. In addition to this, we have created a fourth user level called "super user." The super user has both administrator and supervisor rights. This creates a "true" administrator that can perform any function on the system.

Enhanced Reporting Features

Although all the features discussed above are very important for an identification system in the pharmaceutical industry, there is nothing more important than its ability to produce sound and accurate results and to report them in a clear manner. The V2C does just that. The identification reports can be set up to print automatically or manually. We have it set up manually, so the user performing the identification has an opportunity to review their results. This puts their electronic signature on the printout and manual signing is not required. The report contains all the usual suspects, such as date

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Upcoming Events

May

- May 14th—15th **PMF Validation Conference**
  Location: Ft. Worth, TX
  website: http://www.highpeaks.us
- May 22nd—25th **Annual ASM Meeting**
  Location: Toronto, Canada
  WebSite: www.asm.org

June

- June 4th—5th 2007 **PMF Cosmetic Microbiology Conference**
  Location: Newark, NJ
  website: http://www.highpeaks.us

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: 2,296
Number of Countries: 64
Number of Messages Last Month: 361

PSDGList (Pharma Stability Discussion Group):
Number of Subscribers: 926
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

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and time the ID was completed, the date and time of the completion of the ID, the printing of the report and the date and time stamp of the electronic signature. It gives the final identification with a percent probability, the biochemical details, which contain the results for all wells in the card and the bionumber, which is determined by the biochemical details. The report also contains the confidence level, which can range from "excellent" to "low discrimination." Low discrimination is a result given when the V2C can't decipher between two or more organisms. In the event of a "low discrimination" result, the V2C will suggest auxiliary tests that could allow for a separation of those organisms. Some of the more frequent tests we've had to perform are urease, maltose or mannitol. Sometimes a Gram stain may be required to check for chain formations or spore shape. All in all, this is very helpful with those organisms that the V2C may have some problems identifying.

Enhanced Database

As discussed above, the V2C has increased the speed with which the identifications are completed. It also has an increased database as compared to the older models. Some of the improvements that stand out the most are its ability to identify microorganisms of the genera *Bacillus* and *Methylobacterium*. All were difficult to complete using the older Vitek or other identification systems (1, 2). In addition, from personal experience the Vitek Legacy couldn't identify *Microbacterium species* very well either. Now these isolates are routinely and quickly identified. The only problem, as most industrial microbiologists may know, is getting *Methylobacterium* to grow on agar plates to enable inoculation. One thing the V2C cannot do is identify Gram-positive irregular rods. However, a new ID card is said to be in the making by bioMérieux that will fill this void. There is also a card for fastidious genera, but since we do not use it I can't write much on that feature.

Overall, the success rate of the V2C has drastically improved as compared to bioMérieux's older systems. Less unidentified results means less re-runs, which translates into money saved. Furthermore, the ability to differentiate similar organisms with auxiliary tests allows for more identification work to be completed in-house, which also saves money on contract lab fees. Being cost-effective is an area where being a phenotypic system vs. a genotypic one is beneficial to the user. The argument is made by many microbiologists that genotypic systems are better. However, if the intent of your lab is to provide efficient results, do it quickly and spend less per each identification, the V2C is the workhorse you need.

In closing, the V2C is an excellent microbial identification system for the pharmaceutical industry. It

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satisfies the need to produce accurate and rapid results, without any compromises on the compliance aspect of the system. It has been a great asset to our lab and has saved us money. This may sound like an advertisement, but it is merely an experience of a satisfied end user.

References:

1. Odumeru, J. A., et. al., Evaluation of Accuracy and Repeatability of Identification of Food-Borne Pathogens by Automated Bacterial Identification Systems. Journal of Clinical Microbiology. Apr. 1999, p 944-949
2. Klingler, J. M., et al., Evaluation of the Biolog Automated Microbial Identification System. Applied and Environmental Microbiology. June 1992, p. 2089-2092.

About the Author

Max Dolgopyat was born in Kiev, Ukraine in 1978. He immigrated to the United States in 1990 living in various cities along the eastern seaboard. He now lives in Middletown, PA with his wife and works at ImClone Systems Incorporated, where he has been for the last 6 years. Max received his BS in Biological Sciences from Rutgers University in 2000 and his MS in Microbiology from Seton Hall University in 2005. Max is a supervisor for the Microbiology Quality Control Department and has spent a lot of time working with microbial identifications using methods such as the Vitek Legacy, Vitek 2 Compact, Biolog, API strips and various supplemental tests. This includes the validations of the Vitek 2 Compact and the Biolog instruments.

The 2007 Validation Conference is Coming!

The PMF Validation conference this year returns to its roots. The first conference was held in the DFW metroplex, and PMF returns this year to a conference hosted at The Renaissance Worthington Hotel in Sundance Square, downtown Fort Worth.

This is a return to beginnings in more ways than one. The first conference dealt heavily in the statistics of microbial validation design, we return to this theme in 2007 and expand to include current issues in validation of alternate microbiological methods.

To this last point we are fortunate to have Dr. Bryan Riley of FDA presenting at this year's conference. Dr. Riley is certified by CDER as a reviewer for Process Analytical Technology and was a member of the review and inspection team for the first submission approved as part of the FDA's PAT initiative. Dr. Riley has been closely involved in the initiative to encourage the pharmaceutical industry to adopt rapid microbiology methods and has given numerous presentations regarding rapid methods.

In addition to Dr. Riley, Dr. David Porter will also be discussing validation of alternate microbiological methods. Until recently Dr. Porter was Director of the General Chapters Group, Drug Standards Development at the United States Pharmacopeia. The conference is rounded out by Dr. Scott Sutton. Drs. Porter and Sutton, while familiar with USP, cannot present a USP position on topics of the meeting but will present information and interpretation based on their expertise.

The conference will cover questions of microbial validation at a fairly rapid pace for the uninitiated. Participants are encouraged to review USP chapters <1117>, <1223> and <1227> as well as the FDA's PAT and Compatibility Protocol guidances prior to the meeting.

Come Join PMF in the Texas sun for some fine food, music and discussion!


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The chapter reinforces the compendial preference for the "seed lot technique" in culture maintenance. Critical to this is the need to go into your containers of stock culture only once, and in restricting the number of passages. Now, it must be stated that there is nothing magic about the number 5. This number gained popularity in the compendia through its use in the Sterility Test, and has been maintained for consistency. The point to the practice is that a careful lab will safeguard the purity and identity of their stock cultures by limiting the potential for "drift" due to excessive transfers.

Maintenance of Laboratory Equipment

This section was included more for the sake of completeness than because of concerns peculiar to the microbiology laboratory. Most lab equipment in the microbiology laboratory is subject to the standard validation practices of IQ, OQ, and PQ. As is common, periodic calibration/maintenance may be required for the particular equipment based on its nature, and performance verification checks should also be performed regularly. The frequency will depend on characteristics and use of the equipment.

Laboratory Layout and Operations

The need for this section stems from the concern that too few facilities understand or plan for the separation of samples from a microbiological perspective. The success of a laboratory can be enhanced by the thoughtful separation of samples likely to have contamination from those that are expected to be sterile.

Training of Personnel

The chapter states plainly what should be common sense in recommending that microbiologists and managers in the pharmaceutical support lab should have academic training in microbiology or allied health sciences. This recommendation is in line with current best practice for biosafety as laid out in the 5th Edition of the Center for Disease Control's (CDC) manual "Biosafety in Microbiological and Biomedical Laboratories (BMBL)." (4)

In addition to the recommendation that the microbiology staff have studied a relevant subject while in school, the proposed guidance chapter points out a fundamental link between training and the unit's SOP system. It recommends that the SOP system should be comprehensive and serve as basis of the training program. This proposal also recommends that performance assessments be done periodically and should demonstrate competency in core activities of the lab.

Documentation and Maintenance of Laboratory Records

Like the section on equipment maintenance, this section was included only for the sake of completeness.

Interpretation of Assay Results

This section was initially entitled "OOS Investigations" it was renamed "Microbial Data Deviation Investigations" out of deference to the work underway by a PDA task force. However, during the writing process it became clear that the scope of this section was broader than merely investigations, and so the current title was settled upon almost by the process of elimination.

A discussion of the inherent variability of microbi-

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ological data was necessary in this chapter. One view of good laboratory practices could be structured around determining practices that minimize variability in the microbiology lab. However, because we are dealing with such low numbers on plates (frequently less than 20 CFU/plate) and the real opportunities for human error in tests that may run over a month to completion, the microbiologist must always be aware of the role that random chance has in the data and be on guard against over-interpreting the results of a study.

This section of the proposed guidance document is intended to be both a discussion of the limitations of compendial test methodologies and a guide to developing methods of investigating test failures. It discusses the difference between a test that has failed, a test that should be invalidated and a test that should be repeated for confirmation.

Summary

The proposed chapter <1117> Good Microbiological Laboratory Practices" was developed in response to repeated requests from industry for guidance in this area. This chapter is meant to provide guidance to workers and to regulators in evaluating the operations of the QC microbiology lab.

This article is an updated version of an article by the author that originally appeared in the *PMF Newsletter* vol. 11 no.2 (2004).

References

1. USP. 2006. <1117> Microbiological Best Laboratory Practices. *USP 29 Suppl 2* pp. 3804-3807
2. USP. 2003. <1117> Good Microbiological Laboratory Practices. *Pharmacopeial Forum* 29(3):842-850.
3. USP. 2004. <1117> Microbiological Best Laboratory Practices. *Pharmacopeial Forum* 29(3):1713-1721
4. CDC. 2007. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm released to the web in February, 2007

New Flow Cytometry Application for the Rapid Detection of Yeasts and Molds

AES CHEMUNEX, has announced the launch of a new application using flow cytometry for "Presence/Absence test of Yeasts and Molds in Personal Care and Non-sterile Pharmaceutical Products". By combining the 24 hours presence/absence test for Bacteria with the 2 days presence/absence test for yeast & molds, AES CHEMUNEX provides a complete solution for rapid microbiological detection to the Pharmaceutical and Cosmetic industries that now enables companies to release final products 3 to 5 days earlier compared to traditional methods.

The presence/absence determination is based on a preincubation step using a specific culture media to reach the required sensitivity level, followed by the labeling of viable microorganisms using a viability substrate. The detection and counting of labeled microorganisms are performed in real time as they pass cell by cell in front of a laser through the flow cytometer quartz flow cell.

For the routine implementation of these applications, AES CHEMUNEX proposes to quality control laboratories a range of flow cytometry systems (D-Count, BactiFlow ALS and BactiFlow) with different levels of automation up to 50 samples per hour and a fast track validation support to facilitate the introduction of the alternative method for control of microbiological quality.

In addition, this technique may also offer the potential to detect and to enumerate in minutes the microbial bioburden in materials such as process water through direct counting applications.

The quality control laboratory can now provide microbiological results within minutes or hours after sample collection. Sensitive and rapid control of microbial contaminations throughout the manufacturing process provides real benefits to industrial producers such as the reduction of quarantine time, the decrease of inventory costs, a faster response to contamination incidents.