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PMF NEWSLETTER

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- High Peaks Associates, Inc. (http://www.highpeaks.us)

to the continued success of the *PMF Newsletter*

The Summer Issue

Scott Sutton, Ph.D.
Editor, PMF Newsletter

The summer is a time where it is always a bit more difficult for me to focus on the task at hand. I would prefer to look forward to my upcoming vacation, reminisce about my recently completed vacation, or just go outside and enjoy the sunshine and lack of snow. I assume that many share this difficulty, and so this issue we present a lighter book for review, suitable for summer reading. Good to Great by Jim Collins is not a scientific textbook, but rather a "management" book. We recommend it anyway.

Important Links:

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

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

In the ongoing series on microbial identification technologies we present a review of the technology know as FAME (fatty acid methyl esterase) analysis by GC chromatography. This is a mature technology, one many use in the pharmaceutical and personal products industry.

The confusing topic of inoculum preparation is considered. This should be a very straight-forward procedure, after all it is required for almost every test. However, it never hurts to review different methods of a common practice, and this article examines the topic from the perspective of the Antimicrobial Efficacy Test. This is the most confusing area of the topic, as the pharmacopoeia present conflicting instructions on the issue.

Finally, the PMF Fall Forum is coming - reserve October 9th and 10th! A preliminary list of topics and speakers can be found on page 10.

Scott Sutton, Ph.D.
scott.sutton@microbiol.org

Don't miss the PMF Conference:
Bacterial Endotoxin Summit
http://www.highpeaks.us/2006/BES

Microbial Identification by Automated Fatty Acid Methyl Esters Analysis

Jeff Little
Vectech Pharmaceutical Consultants

The Sherlock* Microbial Identification System (MIS), is an automated rapid microbial identification system introduced in 1985 by MIDI Inc. The system works by gas chromatographic (GC) analysis of fatty acid methyl esters (FAME), and the system is composed of the MIS software and libraries, along with an Agilent Technologies 6850 or 6890 model GC. During the sample preparation, the fatty acids from an unknown microbe (fatty acids from 9-20 carbons in length) are extracted and converted to their respective FAME. The fatty acids from the unknown microbe are then separated by the GC (represented by a chromatogram), as each fatty acid peak in the chromatogram is named by the MIS software (using pattern recognition algorithms based on the fatty acid composition within the cellular membrane), and the complete fatty acid profile fingerprint) is compared to the MIS libraries of known microbes used for identification (9). The MIS software controls the analytical operations of the Agilent GC, names the unknown fatty acid peaks, and identifies the organism by comparing the entire fatty acid profile to the MIS libraries.

The MIS allows for the use of various libraries. A current total of 4471 species are shared by the libraries (with some duplication of species between libraries). The types of libraries and composite number of species present in each library are as follows:

- 16S DNA bacteria/mycobacteria = 1,300**
- 28S DNA fungal/yeast = 1,100
- (GC-FAME) Aerobic bacteria / Environmental= 695
- (GC-FAME) Aerobic bacteria / Clinical = 430**
- (GC-FAME) Aerobic mycobacterium = 25
- (GC-FAME) Aerobic bioterrorism = 6
- (GC-FAME) Anaerobic bacteria = 725
- (GC-FAME) Yeast = 190

An additional 400 species are to be included by the end of the 2006 year (indicated libraries marked above with double asterisks); as well as, an additional 300 aerobic species scheduled for mid 2007 (a majority of which are *actinomycetes*). MIDI also enables its users to create a custom library for their specific identification needs.

With the use of a dual tower 6890 series gas chromatograph and the appropriate placement of samples within the loaded sample tray/turret(s), up to 96 samples can be analyzed per eight hour shift. Sample preparation time averages about 5 minutes per sample, at under $3.00 per sample using MIDI's *Rapid* methods (*Standard* and *Sensitive* methods may also be used – contact MIDI for more details).

Taking into consideration the cost per sample, the limited amount of time needed by the operator, efficient design of the graphical user interface, and the highly defined objectivity of the organisms named, microbial identification using gas chromatographic fatty acid methyl esters (GC-FAME) analysis is attractive for many applications. In addition to the FAME-based identification technology, MIDI also offers a DNA sequence-based identification software option called *Sherlock DNA*. The *Sherlock DNA* software options enable the operator to input sequenced 16S rRNA and 28S rRNA data information into the *Sherlock* software to generate a combined GC-FAME/DNA identification report using phenotypic and genotypic results for a single sample.

However, as in all technique-driven assays, the operator must be knowledgeable of the required conditions and proper techniques used throughout the process to obtain reliable, reproducible results for each sample load. Below are some important areas that should be stressed to the operator(s) and management.

Important factors where errors can potentially occur:

Media used:

Due to the numerous different kinds of media available in the marketplace today, and with the multiple growth constraints required by each selective media type (1, 2, 4), MIDI has required the use of specific media types and growth conditions outlined in their procedure for specific types of organisms. In doing so, MIDI has eliminated the slight variations of growth and cell integrity observed when similar organisms are grown and harvested on differing media platforms (9). Furthermore, the MIS libraries were created using the same media and growth conditions required during the sample preparations stage to ensure con-

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Measurement of Cell Concentration in Suspension by Optical Density

Scott Sutton, Ph.D.
Vectech Pharmaceutical Consultants

A common issue for the microbiology lab is the determination of starting inoculum concentration. If the inoculum concentration is determined by plating, the inoculum is several days old before use. This essay describes the use of turbidity to estimate microbial concentration in a suspension, using the Antimicrobial Efficacy Test as the example.

Determination of Inoculum for the AET

The compendial antimicrobial efficacy test (AET) requires inoculation of the product with microorganisms to a final concentration of approximately 10^6 CFU/mL. Although this seems to be a minor point, it does serve to illustrate some of the inherent difficulties in microbiological testing and the need for experienced and academically trained microbiologists to head the laboratory.

Let's look at the compendial guidance. The Pharm Eur (1) instruction on preparing the inoculum for the AET states:

> "To harvest the … cultures, use a sterile suspending fluid … Add sufficient suspending fluid to reduce the microbial count to about 10^8 microorganisms per milliliter…Remove immediately a suitable sample from each suspension and determine the number of colony-forming units per milliliter in each suspension by plate count or membrane filtration (2.6.12). This value serves to determine the inoculum and the baseline to use in the test. The suspensions shall be used immediately."

There are, of course, two problems with these instructions. The first is that the technician is instructed to use an inoculum of about 10^8 microorganisms per milliliter and then instructed to determine this by plate count. Colony forming units (CFU) and cells are two different measures and this will inevitably lead to difficulties as the unfortunate lab worker cannot guarantee the number of cells in the suspension, only the number of CFU found. However, we can accept the scientific inaccuracy as the numbers will generally work out. The more serious problem is the instruction to use the plate count CFU for determination of the inoculum for the test, and that the suspension shall be used immediately. This quite frankly cannot be done. If you use the suspension immediately, the plate counts are unavailable, if you use the plate counts to set the inoculum, then the suspension is at least a day old.

Contrast these instructions with those in the USP (2) for the same exercise:

> "To harvest the … cultures, use sterile saline … Add sufficient … to obtain a microbial count of about 1 x 10^8 cfu per mL…[Note: The estimate of inoculum concentration may be performed by turbidimetric measurements for the challenge organisms. Refrigerate the suspension if it is not used within 2 hours].
> Determine the number of cfu per mL in each suspension …to confirm the initial cfu per mL estimate. This value serves to calibrate the size of the inoculum used in the test."

These USP instructions have the advantage of being physically possible to perform, an obvious advantage to the lab worker. However, the turbidometric measure of the cells is also only an approximation of CFU. Thus the instruction to confirm the numbers (after the test is underway) with the plate count is an important control on the test.

This article will explore the turbidometric approximation for cell numbers, and important controls on the process as well as potential pitfalls to the method.

Theory

Light scattering techniques to monitor the concentration of pure cultures have the enormous advan-

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sistent microbial identification. The operator(s) must be capable of understanding and accurately interpreting the growth expectations for each organism, based on the visual identification of characteristics created by the growing constraints influencing the organism. Due to the level of observation and experience required to provide reliable and consistent growing conditions for all organisms, subtle variations should be monitored, and reported, as indicated within the appropriate standard operating procedures (SOP).

Temperature:

Temperature is one of the key factors that affect the growth conditions of any organism (3, 5, 8). Whether extremely cold or warm environments (11), organisms adjust to their environment to compensate for the constant change of cell membrane fluidity required by the cell to maintain cell integrity and obtain nutrients that are required for survival and development (6). Each operator needs to be fully aware of the discrepancies and possible variation created due to the slightest fluctuation of temperature which can endanger the reliability and reproducibility between samples. Any deviation from MIDI's required growth conditions could greatly affect the fatty acid profile generated.

Colony age:

The physiological state of the cell is a critical parameter of the test as the MIS analyzes the fatty acid profile of the cell membrane, a characteristic that changes with the age of the colony (10). The log phase of growth is required for proper identification by the MIS as the cell membrane fluidity, created by the number of cis- and trans- bonds located throughout the fatty acids can be controlled by this method. Only with the operator's understanding to the impact of the colony's age of the growing cell and how these subtle growing constraints can influence the integrity of the cell membrane, will the MIS provide reproducible results.

Use of glass or Teflon covered materials:

The use of clean and sterile materials that do not influence the test results is critical. The designed sensitivity of the MIS and the remove the risk of generating contaminate peaks during the GC-FAME analysis (especially from plastic caps and rubber stoppers) only increases this need. The use of glass, or Teflon coated materials is recommended. Glass and Teflon are not directly influenced by the chemicals used during the preparation sequence, and alleviate any concern for contamination influenced directly from the materials used during the process.

Operator variance:

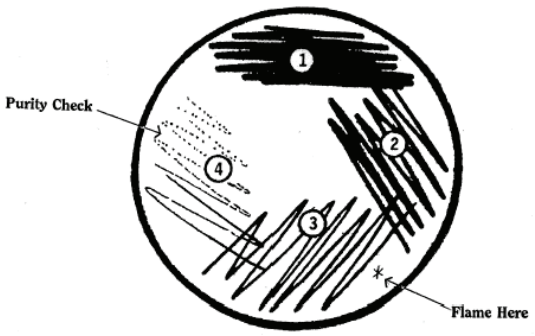
The proper preparation of the sample is a key factor in obtaining consistent results on the MIS. All project personnel should be familiar with the essential components and factors that contribute to the MIS results as described in this essay or provided by the vendor. In addition to accurate pipetting of the needed solutions, and the precise addition and mixture requirements, any type of variations should be adequately tested, validated and documented for reference within the appropriate SOP before using in an identification procedure.

Correct quadrant selection of colony growth:

Relating directly with the colony's age (see Colony Age above), the operator must be familiar with the appropriate quadrant of growth conditions for each sample of interest.

The initial colony of interest is streaked for single-colony isolation by standard methods. Then, a well isolated single colony from that plate is streaked for identification. MIDI recommends the standard four quadrant streaking technique when obtaining a colony to be identified on the MIS, using the third quadrant for most cases (after verification of single, well-isolated colonies in the fourth quadrant). Although the quadrants may vary due to the growing conditions of the colony or the characteristics of the organism itself, the operator should have a standardized operating procedure to aid in the process (6). The four quadrants should be considered in the following manner:

Quadrant one: This quadrant is the first area / quadrant streaked onto the selected media plate from the original source. Being the first area of inoculation, growth constraints increase substantially. As colony growth decreases logarithmically, the colonies begin to



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overrun each other with waste and by-products, while those still trying to promote growth are competing for the remaining nutrients and expansion constraints of the exhausted media substrate. Due to the nutrient depletion and toxic waste production that influences the growing conditions of all nearby colonies, many of the cells are well into the "death phase". Therefore, quadrant one should not be considered for the MIS process.

Quadrant two: Unlike quadrant one, quadrant two tends to be more acceptable for colony selection, but only for slower growing colonies. Within the second quadrant, many of the colonies have reached a stage where the cell growth has reached its plateau. The available nutrients have been depleted and the colonies begin to prepare themselves for their environmental stresses and the reduction of nutrients. Along with the limited cell growth, the by-products and waste begin to accumulate. Although not recommended for most samples, colonies that are considered slow growers may be taken from quadrant two, but the operator should have SOP to aid in the process.

***Quadrant* three:** This quadrant is the standard quadrant of choice when dealing with the conditions of most organisms, as many of the colonies are in the "log / exponential phase" of growth. Quadrant three is considered to be the quadrant where log growth (as seen as a log curve) is occurring for the organism. During the log growth stage, a colony has an abundant supply of nutrients and space available for its expansion and uninhibited growth. The colony has already adjusted to the growing limitations of the media, and has optimized its growing cycle to reflect the unlimited constraints between a balanced growth rate regulated by the overabundance of nutritional components still available from the selected media. Without having the constraints and inhibiting factors to affect the fatty acid carbon chains within the cell membrane as occurs in colonies located in quadrants one and two, quadrant three is the preferred quadrant for reliable results, providing the most stable and reproducible fatty acid compositions (profiles) for a given species.

Quadrant four: The fourth quadrant is typically used only to determine the purity of a streaked colony based on the isolation and separation of the streaking technique. The spacing between the colonies allows the operator to visually observe and identify if there are any residual contaminants carried over from the original source. This quadrant is generally not useful for MIS testing since colonies are still adjusting to the new environment and may be promoting the synthesis of different carbon chains of the fatty acid to create a suitable fluidity of the cell membrane needed for growth.

Proper Interpretation of the Similarity Index:

The technique used by the MIS to compare an unknown organism to the MIS libraries is based on Similarity Index (SI). The SI of the MIS is a numerical value (0.000 to 1.000), which expresses how closely the fatty acid composition of an unknown sample compares with the mean fatty acid composition of the strains used to create the library entry or entries listed as its matches. The automated library search (after each sample analysis) presents the best matches and the associated SI value. This index value is a computer-generated calculation (using pattern recognition software) of the distance between the profile of the unknown and the mean profile of the most similar library entry. Therefore, SI is not a "probability" or percentage, but an expression of relative distance from the population mean.

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An exact match of the fatty acid makeup of the unknown and the mean of a library entry would result in a SI of 1.000. As each fatty acid varies from the mean percentage, the SI will decrease in proportion to the cumulative variance between the composition of the unknown and the library entry. On average, ten or more different strains of a given species are used to create each library entry, to account for the normal fatty acid profile variance within a species. In addition, clinical and environmental organisms of the same species are separated into different libraries because the fatty acid profiles of a given species are different depending on the environment from which they were grown.

Understanding how to interpret the numerical SI value is crucial for proper identification of the sample. SI values between 0.000 - 0.299 denote that the sample species is not currently included within the library being used for identification. If the SI is between 0.300 - 0.500 and there is a > (greater than) 0.100 separation between the next closest value(s), the sample is considered to be a reliable atypical species. SIs with a value of 0.500 and higher are considered to be equivalent to the organism listed as the 1st result (highest numerical value), as long as there is still the same > (greater than) 0.100 separation between any additional listed values (7). However, for both SI values that fall between 0.000 – 0.299, and for SI values that have ≤ (equal to or less than) 0.100 of separation, these sample species must be evaluated accordingly. To aid with this data interpretation process, the proper documentation and corresponding SOP(s) should assist the operator with these decisions.

Denoting / labeling of plate nomenclature used by MIS database software:

Although the MIS software is extremely user friendly, enabling the operator to separate out and compare various samples, the operator must understand the naming convention used by the MIS software. The data system is designed to sort and separate file names by the simple addition of a "dash" keystroke between each term of a file name. Each dash that is inserted into the file name indicates to the data system that the information/name prior to the dash is a separate identifier. MIDI recommends going from general information to specific information from left to right (example: Date-time-person-test number-plate-media type-etc.) with a limitation of 42 characters (spaces included). The operator must also remember that each similar entry must match the corresponding entry used for comparison purposes. Even capitalized letters may not be recognized by the data system as the same file name, so it is important for a procedure to be in place to correct for this issue reducing the time and resources needed to find a mislabeled file/sample. Along with the ease and speed of sorting information, the extensive design of the MIS software provides the user with many valuable features that should be evaluated to aid the operator in using the MIS to its fullest capabilities.

Because the MIS recognizes over 100 fatty acids in the cell membrane, and uses qualitative (names) and quantitative (concentrations) fatty acid profiles, MIDI has developed a new *Tracker/Cluster* software option to aid in the process. With the use of the newly developed *Tracker/Cluster* software option, sample tracking can be achieved immediately after completion of the GC analysis using comparative results between the fatty acid profile(s) for individual samples (including unidentified sample strains) and a user defined database.

Due to the flexibility and compatibility of the MIS software options, data reports generated by the MIS can be easily searched, sorted, and evaluated with little to no additional computer software training. This lowers the overhead cost typically required for additional software training, and limits the cost of upgrading or redesigning of the current software infrastructure already in place, while enabling the operator better management of their time for other activities. MIDI uses a polyphasic approach to analyze both GC-FAME and DNA sequenced data into a single report. MIDI Inc. also provides its operator(s) with a collaborative support base which in-

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Internet Address	Description
www.atcc.org	ATCC's site - excellent for tracking name changes of different organisms as well as ordering strains. Also available is a newsletter on cultures and preservation techniques.
http://www.rapidmicrobiology.com/	This site is intended to be a source of useful information for all aspects microbiology testing and related information. A good source for recent press releases by manufacturers.
If you have found an Internet site that contains information of relevance to pharmaceutical microbiology, please let us know.	

Upcoming Events

August

- 10th - **Validation Issues with the harmonized Microbial Limits Test**
  Location: Webinar
  WebSite: http://www.pda.org/webmodules/webcalendar/wcevent.asp?eventid=330
- 23rd - 25th **Environmental Monitoring**
  Location: Washington, DC
  WebSite: http://www.ivthome.com/shop/scripts/prodView.asp?idProduct=1824

September

- 11th - 12th **PMF Bacterial Endotoxin**
  Location: San Francisco Bay Area, CA
  Web Site: http://www.highpeaks.us/2006/BES/
- 11th - 14th **Society for General Microbiology**
  Location: University of York
- 11th - 12th **2006 PDA/FDA Joint Regulatory Conference**
  Location: Washington, DC
  Web Site: http://www.pda.org/webmodules/webcalendar/wcevent.asp?eventid=244

October

- 2nd - 3rd **EDQM Meeting—New Microbiology Chapters of the European Pharmacopoeia**
  Location: Strasbourg
  Website: http://www.pheur.org/site/page_647.php
- 9th - 10th **PMF Fall Forum**
  Location: Rochester, NY
  Website: http://www.highpeaks.us/2006/PMF.Fall.Forum/
- 27th - 28th **PDA Microbiology Meeting**
  Location: Bethesda, MD
  Website: http://www.pda.org/microbiology2006/

Offering In-House Courses on Microbiology/Aseptic Processing:

- The Microbiology Network/High Peaks Associates http://www.highpeaks.us/in-house.htm offers an array of courses in microbiology
- 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: 1,678
Number of Countries: 63
Number of Messages Last Month: 176

PSDGList (Pharma Stability Discussion Group):
Number of Subscribers: 812
Number of Countries: 19

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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tages of being rapid and nondestructive. However, they do not measure cell numbers nor do they measure CFU. Light scattering is most closely related to the dry weight of the cells (3).

Light is passed through the suspension of microorganisms, and all light that is not absorbed is re-radiated. There is a significant amount of physics involved in this, and those interested are referred to optical treatises, particularly those discussing Huygens' Principle (a good choice is Light Scattering by Small Particles by H C Van De Hulst). For our purposes it is enough to say that light passing through a suspension of microorganisms is scattered, and the amount of scatter is an indication of the biomass present in the suspension. In visible light, this appears "milky" or "cloudy" to the eye (3). It follows from this that if the concentration of scattering particles becomes high, then multiple scattering events become possible.

Methods

McFarland Turbidity Standards

McFarland standards can be used to visually approximate the concentration of cells in a suspension. The McFarland Scale represents specific concentrations of CFU/mL and is designed to be used for estimating concentrations of gram negative bacteria such as *E. coli.* Note that this estimate becomes uncertain with organisms outside the normal usage as different species of bacteria differ in size and mass, as do yeast and mold. Use of this method would require calibration and validation.

McFarland Standards are generally labeled 0.5 through 10 and filled with suspensions of Barium salts. (Note - latex bead suspensions are now also available which extend the shelf life of the material).

The standards may be made in the lab by preparing a 1% solution of anhydrous $BaCl_2$ and a 1% solution of H_2SO_4 – mix them in the proportions listed in the table. They should be stored in the dark, in a tightly sealed container at 20-25°C and should be stable for approximately 6 months (4).

The advantage of the use of these standards is that no incubation time or equipment is needed to estimate bacterial numbers. The disadvantage is that there is some subjectivity involved in interpreting the turbidity, and that the numbers are valid only for those microorganisms similar to *E. coli*. In addition, the values are not in the appropriate range for the AET inoculum and so further dilutions may be required.

Approximate *E. coli* concentrations on McFarland

McFarland Scale	CFU (x10^6/mL)	1% $BaCl_2$/ 1% H_2SO_4 (mL)
0.5	<300	0.05/9.95
1	300	0.1/9.9
2	600	0.2/9.8
3	900	0.3/9.7
4	1200	0.4/9.6
5	1500	0.5/9.5
6	1800	0.6/9.4
7	2100	0.7/9.3
8	2400	0.8/9.2
9	2700	0.9/9.1
10	3000	1.0/9.0

Spectrophotometer

The spectrophotometer method measures turbidity directly. The best case (*i.e.* most sensitive) would be to have a narrow slit and a small detector so that only the light scattered in the forward direction would be seen by the detector. This instrument would give larger apparent absorption readings than other instruments.

Scale

As should be obvious, each spectrophotometer used must be independently calibrated for use in estimating microbial concentrations. Not only is the apparent absorption affected by the width of the instrument's slit, the condition of the filter, and the size and condition of the detector, but also each time the lamp is changed the calibration needs to be repeated as different bulbs may vary in total output.

The correlation of absorption to dry weight is very

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good for dilute suspensions of bacteria (5), and this relationship seems to hold regardless of cell size (although the relationship of absorption to CFU does not). However, in more concentrated suspensions this correlation (absorption to dry weight) no longer holds. The linear range of absorption to estimated CFU is of limited scope and for this reason the calibration study must demonstrate the linear range of the absorbance *vs* CFU values and the relevant values.

Procedure

As there are a variety of different instruments, there cannot be one single procedure. In general, the spectrophotometer can be set at a wavelength of 420 – 660 nm. This wavelength *must* be standardized and may need to be adjusted specifically to the material being tested. Different vegetative cells, bacterial spores and spores of *Aspergillus niger* may not have the same maximal absorbance wavelength.

It is important to have the cells in known physiological state of growth. That is to say, as the cell size varies with phase of growth (lag, log, stationery) the approximate relationship between absorbance and CFU will also vary. A recommended practice might be to pass a single well-isolated colony twice on overnight cultures surface streaks from the refrigerated stock, harvesting the rapidly growing culture from the second passage for preparation of vegetative cells. This also will serve to minimize a source of variability for the AET (6).

A second source of concern might be the cuvette used for the measurement – care must be taken to maintain the correct orientation of the cuvette, and to protect it from damage that could affect the passage of light. Finally, it is necessary to blank the spectrophotometer (adjust the absorbance reading to zero) using a standard, either water or the suspending fluid, and maintain this practice.

A final area of concern is the tendency of some micoorganisms to settle, and to clump on storage. There are some microorganisms (the naturally competent *Bacillus subtilis* springs to mind) that will aggregate in suspension under some conditions. Most organisms in heavy suspensions will settle if left undisturbed. Therefore, the procedure should include a visual inspection and a vigorous vortexing immediately prior to the spectrophotometer reading.

Calibration

It must be stressed that this calibration should be done for all organisms. The size of the organism, any associated pigments, the preparation of the suspension, and other factors all influence the readings. This calibration study should also be rechecked after changing the bulb on the light source, and should be reevaluated throughout the life of the light bulb.

The calibration itself is simple to perform. Prepare a concentrated solution of the organism, grown under the conditions that will be used for the test. Make a series of dilutions to cover the range of absorption measurements of interest; 5 to 8 dilutions are recommended. Immediately take the spectrophotometer readings in sequence, and then take a confirmatory reading of the first in series to confirm that no growth has occurred. The dilutions are then immediately plated for viable count (serial dilution of the suspensions will be necessary). Graph the relationship between the absorbance and the CFU/mL after the plate counts are available and use values in the linear range of this graph.

As there are several factors that can affect this curve

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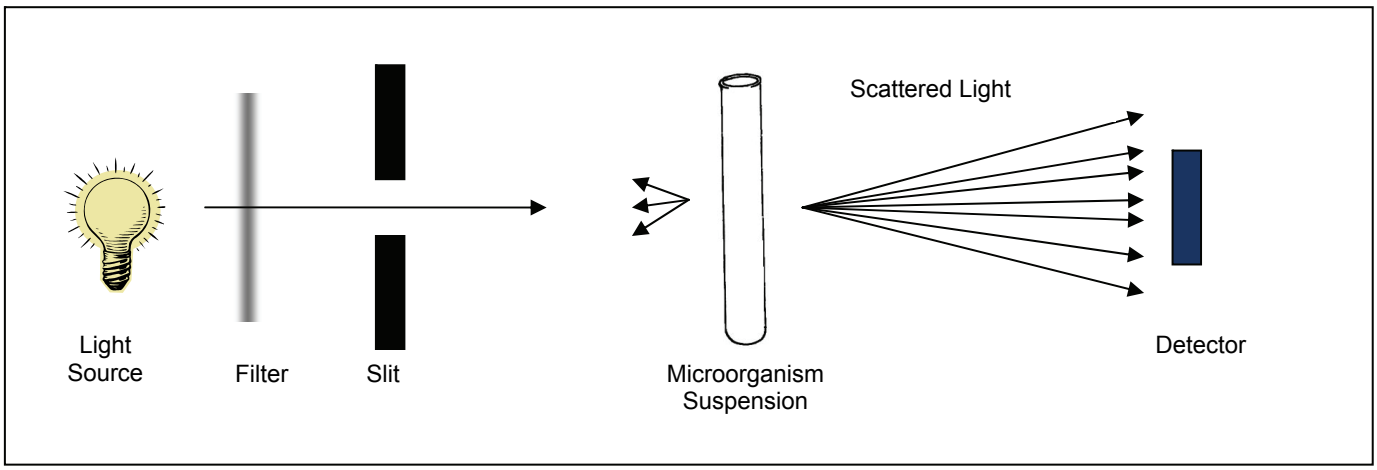

Spectrophotometer

FDA Approval for First Portable Endotoxin Test System

Charles River Laboratories International, Inc. has received FDA approval that allows for the sale and marketing of the first portable endotoxin test system. The patented Endosafe-PTS™ is used to detect endotoxin contaminants in drugs, medical devices, biological products and other unique applications including NASA spacecraft missions. The PTS is a revolutionary technology that:

- is a portable handheld system that can be used for point of use testing
- provides quantitative results in 15 minutes rather than current methods which take from 45 minutes to several days
- requires minimal technical training – just insert the PTS cartridge, add sample and read quantitative results

The Endosafe-PTS™ system can accelerate the discovery and development of new drugs by offering quick and easy endotoxin contamination results, thereby saving pharmaceutical companies, medical professionals and scientists both time and money in the manufacturing process.

Real world applications for the PTS system include: Medical devices & injectable drugs, Dialysis clinics, Nuclear medicine ,Hospitals, Stem cell research and Planetary protection

The PTS utilizes disposable cartridges that are pre-loaded with reagents and a portable, handheld reader to provide quantitative endotoxin amounts. Because of its portability, the PTS allows for testing to be performed at the point of sample collection. In addition, the simple one-button operation requires minimal technical training and the data can be stored in the PTS, downloaded to a computer and/or printed.

NASA recently used the Endosafe-PTS™ to monitor the environment for microbial contamination during the construction of the Mars exploration rovers "Spirit" and "Opportunity". PTS units are scheduled to be flown to the International Space Station to perform environmental monitoring for microbial contamination. Future applications of PTS may include detecting signs of microbial life on Mars.

Source: Charles River Laboratories as posted 7/24/06 on rapidmicrobiology.com

The 2006 PMF Fall Forum is Coming!

The 2006 PMF Fall Forum will be October 9-10 in Rochester, New York. Come and discuss current topics with the leaders in the field—those who publish are the invited speakers!

Speakers and Topics include (to date):

- Culture Collections and Cryopreservation; Liz Kerrigan (ATCC)
- Harmonized Microbial Limits Tests; Scott Sutton (PMF)
- Analysis of EM Data; David Hussong (FDA - invited)
- Environmental Monitoring for non-steriles; Jeff One (Abbott)
- Strategies for Managing Environmental Monitoring Investigations; Dilip Ashekar (Amgen)
- Discussion of Part 9000, Microbiology, in Standard Methods for the Examination of Water and Wastewater; Margo Hunt (EPA)
- Aseptic Process Simulations (media Fills) Program at a Parenterals Pharmaceutical Company; Roxanne Robles-Torres (Wyeth)
- What Is Disinfectant Validation? Jose Martinez (JEM Consulting)
- Disinfectants and Cleaning; Art Vellutato, Jr. (Veltek)
- Microbiology Training Programs; Jackie Hewitt (Luitpold Pharmaceuticals)
- Training for Aseptic Processing Environments; Anne Dixon
- Biological Indicators; Jeanne Moldenhauer (Vectech)

Good to Great by Jim Collins

I normally reserve my enthusiasm for "management" books to roughly the same level as my enthusiasm for "healthy diet" books. However, I am indebted to the person who recommended to me the book Good to Great by Jim Collins (HarperCollins Publishers, New York, NY 2001. ISBN 0-06-662099-6). Unlike most in the genre, this author headed a research team, collected data, and then used the data to generate his conclusions. This is the opposite of most management books I have endured and an approach I found amazingly interesting (a management book that first collects data, then draws conclusions!). I am going to break with the general rule and discuss a book that is not directly related to microbiology as this book has made such an impression on me.

The study plan was relatively simple. "Good-to-great" companies were identified by looking at the Fortune 500 companies and analyzing their stock prices relative to the market. To be in the short list, the company needed to be a good performer, then have a transition point after which the company needed to demonstrate a cumulative stock return of at least 6.9 times the market average over 15 years (to weed out the stellar but unsustainable performances).

Having identified the "good-to-greats" the research team then identified comparator companies. As the author states: "The crucial question in our study is *not,* What did the good-to-great companies share in common? Rather the crucial question is, What did the good-to-great companies share in common that *distinguished* them from the comparison companies?" Two sets of comparison companies were chosen. Direct comparators were in the same industry and had similar size and opportunities to the good-to-great but showed no remarkable growth. Unsustained comparators made a short-term shift to stellar performance but did not maintain it.

The team then collected all information they could acquire on the good-to-greats and their comparators (articles for past 50 years, interviews, annual reports, acquisitions, etc) and coded all information and asked the question "What's different between the two (three) groups of companies?"

This article is really too short to go into their findings with the detail they deserve, but *on the basis of the data* the author proposes the following principles of good-to-great performance:

Level 5 Leadership – Rather than the super-star CEO with a mega-salary, the first principle seemed to be a blend of personal humility and professional will. The primary focus of the CEO and leadership team was the company, not building a new headquarters with corner-offices for all the VPs.

First Who … Then What – during the period prior to the transition of "good" to "great", the new CEO did not start out by setting a new vision and direction for the company. Rather he/she focused on getting the best quality people possible to come to work for the company, and cutting out the deadwood (primarily in the executive ranks). The vision developed from the people so assembled.

Confront the Brutal Facts (Yet Never Lose Faith) – The companies had the absolute faith that they would succeed, yet also had the discipline to view the brutal reality of their situation without flinching (or killing the messengers).

The Hedgehog Concept – This label derived from a parable of the hedgehog and the fox. The fox, all fancy and flashy, kept coming up with new ways to attack the hedgehog with the intent of having him for lunch. The hedgehog had one trick, he rolled up in a ball of sharp spikes that thwarted the fox. No matter the new trick of the fox, the hedgehog prevailed. Jim Collins argues that there are three considerations to determining a hedgehog strategy:

- What are you deeply passionate about?
- What can you be the best in the world at?
- What drives your economic engine?

Where these three considerations intersect should determine your core business. One trick, do it better than anyone else.

A Culture of Discipline – This is not chain-of-command "protect your boss and do as you're told" kind of discipline. Rather it is a corporate culture generated by the association of individuals who are each disciplined and working for the common goal. Bureaucracy, in this view, exists to impose control on those unable to discipline themselves. Bureaucracy can be minimized if the culture supports individual accountability and discipline.

Technology Accelerators – The companies never used a new technology as the primary means to ignite a change in performance. Rather they used new technologies as a tool and were frequently pioneers in the application of new technologies.

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The Flywheel and the Doom Loop – This principle is meant to convey the futility of orchestrated programs. The good-to-great companies changed more gradually than expected, like a flywheel that is slow and laborious to get going, but once momentum builds can power many things. No "breakthrough moment" (except perhaps in hindsight). The major characteristic here is consistency. The comparators were partial to grand initiatives. When the first didn't work, they may reverse directions, starting a new grand initiative. Each new CEO to the company would minimize the old initiative in favor of his own, moving on in a "Doom Loop" of unfulfilled expectations. This doom loop had two major versions as revealed in the study: misguided use of corporate acquisitions (acquisitions outside the core business of the company designed to create the appearance of growth) and the selection of leaders who undid the work of their predecessors.

The final chapter of the book Collins devotes to a discussion of how this study relates to his previous book (Built to Last with Jerry Porras). I won't go into that topic here; I haven't read Built to Last and could not really comment on it. However, I have worked for some excellent companies, and the findings of this study resonate with what I have seen practiced.

Why review a management book in a newsletter devoted to microbiology? As I hope you can see, the principles described in this book are as much leadership principles as a roadmap to corporate wealth. The leader should be self-effacing, encouraging others to succeed. The leader should focus on building the strongest team possible, should encourage simplicity in goals, consistency, discipline and accountability. These work as well for the lab manager as the CEO.

If you have a chance, take some time out and at least scan the book. Each principle has a chapter devoted to it, expanding the concept in a very readable manner. In addition, each chapter has a 1-2 page summary highlighting the main message of the chapter. For those who take the time to read the entire book, however, you will find much to think about as you go back to work.

Scott Sutton

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Note: Due to repeated requests for ordering information, this review (and the newsletter as a whole) contains links to purchase books on-line. I hope you find this feature useful.

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(quality of lamp output, size of slit, condition of filter, condition of detector, microorganism characteristic, *etc*) this calibration should be confirmed when the conditions of the assay change.

Conclusions

The use of optical density to estimate CFU in a suspension is possible, if basic precautions are taken. It is important to control:

- The physiological state of the organism
- The species of the organisms (*i.e.* don't calibrate the instrument using *E. coli* and expect the numbers to work for *Candida albicans*)
- The nature and condition of the equipment.

Despite the inherent inaccuracy of the method, if the procedure is adequately controlled and calibrated the estimation of microbial numbers by optical density (either by McFarland Standards or spectrophotometrically) is sufficiently accurate for use in preparing inocula for QC testing and offers the overwhelming advantages of being rapid, low cost and non-destructive.

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cludes the MIS, its knowledgeable team of microbiologists and software developers, various software options, and comprehensive microbial libraries. This is provided as part of the training and validation expected by the self-sufficient laboratory. By reducing the workload and providing a fast efficient timeframe toward a project's completion, the MIS has the ability to reduce overhead by using the cost per sample count (for GC-FAME) of only a few dollars, yet providing reliable and reproducible analysis within minutes.

However, as with any automated rapid-micro system, only with the proper training and comprehension of how the Sherlock Microbial Identification System software capabilities work, at all levels of technical application and lab management, can the data generated by the MIS provide the useful information. One must understand not only, the complete sample preparation process, but the environmental controls and processing factors that could influence the profiles generated for analysis.

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