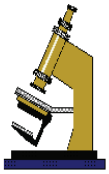
PMF
Pharmaceutical Microbiology Forum



PMF NEWSLETTER

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In This Issue:

Vectech
HPA High Peaks Associates, Inc.

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

to the continued success of the *PMF Newsletter* Newsletter

Basic Microbiology

The central theme of this newsletter is basic microbiology in the pharmaceutical, personal products and medical device industries. This focus is very clear in this month's issue of the newsletter. At a time where many decisions are being made based on an estimation of what will get approval easiest, or what is the least expensive way to complete a study, we also need to go back to basic principles of microbiology in our work. That is not to say that product approval and unit cost are not important - we are, after all, working to support our companies and clients. However, exclusive focus on the "cost" of microbiology is false economy, penny-wise but pound foolish. It seems we always have time to re-do a test, but it is sometimes difficult to find the resources to do it correctly the first time.

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

There are two articles in this month's newsletter that stress this point, although in different ways. The first is an article on water system alert and action levels by TC Soli. Dr. Soli stresses the need for good science in setting control levels for your system, and provides some examples of how to get this done in a manner that should find acceptance with both management and regulatory agencies. The second article is a review of the gram stain. Of little practical interest to those out of the lab, it is critically important to most microbial identification methods. It is also one of the most poorly understood and improperly controlled assays you are likely to see in visiting different laboratories. The article provides a review of the methodology, the areas of the test most open to error, and alternatives to the stain method that you might consider.

Finally, we are sad to note the death of Dr. Michael Korczynski. He passed away last month to the sorrow of his family and many friends. Dr. Roger Dabbah provides a tribute to his passing.

Scott Sutton, Ph.D
Editor, *PMF Newsletter*

Purified Water and WFI Alert and Action "Limits"

T. C. Soli, Ph.D.
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Scared Yet?

Ah, those title words send chills up my spine! Why? To me, it's scary. As a consultant, I see a strong correlation between the use of the term Action "Limit" (instead of Action "Level") and the adoption of what some firms consider to be the USP-recommended Action Levels of 100 cfu/mL for Purified Water and 10 cfu/100mL for WFI (regardless of their typical system performance), plus no hint of a bona fide water specification, and the use of PCA at 35°C for 48hr – with absolutely no evaluation of alternative methods or even of the suitability of the selected test conditions for their own water systems.

So, why is that so scary? Simple. Though these are pretty common practices, they are simply risky things to do with water systems. It likely puts the user in a powerless position relative to analytical-based microbial control and a weak position relative to compliance, especially when a water system design and/or its age are less than ideal. The above practices also correlate with a firm's GMP philosophy being driven more by minimal "get by" adherence to industry precedent than doing the right things based on an understanding of their water system, its impact on their products and uses, and good science.

Many of those firms may not even have a microbiologist on staff that has an understanding of water system microbiology. Too often, a minimally trained technician who performs microbiological tests is considered the "microbiologist" and expected to advise decision makers in matters where they have even less technical knowledge – hence the firm's reliance on precedent rather than science. In the microbiologist's defense, they may not have a degree in microbiology or any industrial microbiology training or experience that could afford them an understanding of the idiosyncrasies of pharmaceutical microbiology, let alone the microbiology of that dynamic, unstable ecosystem we call a pharmaceutical water system. This ecosystem is affected not only by the available nutrients (McAlister 2002), the composition of pipes (Burkhart et al 1996), the temperature of the distribution system (LaPara et al 2000) and other physical characteristics of the construction and operation of the distribution system, but also by the activities of the microorganisms themselves (Matiny 2003). Fortunately, there are several water consultants who DO understand these things and can train your staff to also understand. Also, there are numerous courses and conferences where this information can be acquired. But chose well – not every water conference/course presenter or consultant is truly a water system microbiology expert.

I'm sure I hit more than a few hot buttons with the above statements. If even only part of this scenario applies to you or your company, do not be offended, defensive, or give up hope. You are actually in the majority, and that carries some weight, but frankly, that does not make it completely excusable. It does not justify continuing to use fallacious concepts and antiquated practices when potentially only a few simple corrections could turn everything around and take the worries out of the water system. All of the associated issues are just too much to cover in a single article, so now that I've gotten your attention, I'll address the title concept and a few related ones in this article and get to the other ones in future articles.

Using the Terms Alert and Action Limits

Though intentioned to be microbial process control terms, they actually imply "specifications". This implication arises because a) there probably are no bona fide water specifications and b) the usage of "limits" in the pharmaceutical product and raw material world means "specifications". Even in USP there are numerous limit tests which are pass/fail attribute tests or specifications for monographed articles. USP has the "Microbial Limits" test chapter <61> which is referred to from many a monograph! So the word "limit" is definitely associated with specifications.

Process control levels should NEVER be used or even implied to be used as specifications or limits. Specifications and limits are pass/fail criteria, so to exceed an action limit could be interpreted to mean only one resulting action – rejection, just like with other raw materials! That is certainly not the intended purpose of a process control level. Process control levels are intended to do what the words state. They are levels established at values just high enough, based on past data trends of your system, to signal that changes may be occurring in the control of a dynamic attribute, such as microbial counts. It can be exhibited by a single high level excursion, but more often it is represented by a pattern of lower level excursions. It usually means that some sort of remedial action is needed to bring this dynamic attribute under better control before it escalates to a level that makes the water unsuitable for use. It DOES NOT necessarily mean that the water is currently unsuitable for use.

Lack of Specifications

Most biopharmaceutical firms do not have such things as specifications for their water. This is somewhat ironic in that every other raw material in their facilities has such specifications. However, it is simply not, for the lack of a better term, the "tradition" to have true

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microbial specifications for raw materials manufactured on site like Purified Water or Water for Injection. One reason you will hear is because of the delay in available microbial count data compared to when the water is used. USP has clearly defined specifications for the attributes of conductivity and total organic carbon (TOC) in their monographs for PW and WFI, but not for a microbial content attribute. Does that mean that pharmaceutical firms should follow suit and also NOT have microbial specifications for their waters? Certainly not!

USP's non-mandatory informational chapter <1231>, Water for Pharmaceutical Purposes, attempts to clarify in the starting paragraphs of the chapter why microbial attributes within its Purified Water and Water for Injection monographs are inappropriate (USP 29/NF 24, 2006). One reason given is because there are so many possible uses of those types of water. This multiplicity of uses is widely recognized (CPMP 2002). Those varied uses likely have different microbial requirements, and some, such as the Purified Water used for laboratory testing, may not require any at all. Listing a specification in the monograph would obligate all users of that type of water to the same microbial requirement. Some water users would be burdened with excessively tight or completely unnecessary microbial requirements (and the system design and maintenance operations to maintain those overly tight microbial levels) and others might need far tighter specifications for their applications. Another reason given for no microbial specifications for PW and WFI is related to the stating of a referee test method which may be inappropriate for the microbial population in any given water system. However, this issue will be addressed in a future article in this newsletter.

The bottom line is that a water specification is most aptly chosen by the user to suit their particular quality needs, but why should a specification be used at all?

To Have Specifications or Not

Problems invariably arise when no value is established as a definitive fitness for use limit, i.e. a microbial specification. In this situation, the Action Level becomes an arguable version of a specification (even when it is not called an "action limit"!) because there is no other definitive fitness-for-use designation. That means that every time an Action Level is exceeded, a product or use impact decision must be made – as though it were a Specification excursion. With that as a consequence of an Action Level excursion, the Action Level is usually not set on the basis of system performance data, but rather at a level so high that it may never be exceeded and is functionally used like a specification.

Unfortunately, in spite of instructive text as to how and why to set meaningful Alert and Action Levels for water (USP 2006), the Maximum Action Levels mentioned in USP <1231> are commonly misused for this very purpose, often in water systems that rarely achieve even 10% of these values. Though this approach certainly minimizes the product impact investigations needed because of water system excursions, it also effectively eliminates the usefulness of that Action Level for any meaningful system control because it is far above microbial levels that could signal the beginning of an impending "out-of-control" condition. By the time this Action Level is exceeded, not only is the system well out of control and correctable only with heroic efforts, it has also been allowed to potentially compromise its suitability for use. Had the impending out-of-control condition been recognized or signaled earlier, remedial measures could have been much less extreme and little, if any, product or use impact investigation would have been needed.

The Optimal Solution

So, instead of avoiding the affects and delays of microbial Action Level excursions on your products and processes by establishing Action Levels that are so high they are irrelevant to process control of your water system, you should establish lower, more relevant process control-related Action Levels plus higher microbial Specifications that reflect your required microbial quality level based on your applications. Though you will not find it codified that FDA officially considers 100cfu/mL and 10cfu/100mL to be maximum specifications for Purified Water and WFI, they certainly functionally consider them to be so, based on 483 citations and other regulatory actions. When you officially establish functionally relevant Alert/Action Levels for process control and Specifications for quality control, you can exceed an Action Level, meaning that some microbial control remediation is needed, without the obligation to investigate product or use impacts if the microbial level is still below the Specification. This actually becomes a winning combination that allows true process controlling levels to be used without the painful and costly product impact investigations. In fact, those kinds of investigations are completely avoidable because the water systems can be sufficiently controlled to preclude exceeding the microbial specifications. Because this approach can eliminate ever having a microbial specification excursion, it should also eliminate any concerns over delayed data availability caused by the incubation period of the test.

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The Gram Stain

Scott Sutton, Ph.D.

Vectech Pharmaceutical Consultants, Inc.
Vice-chair, USP Microbiology Committee of Experts

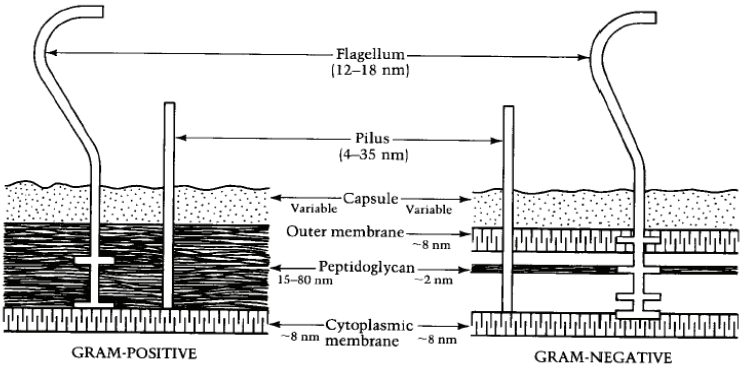
Gram staining is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. The method is named after its inventor, the Danish scientist Hans Christian Gram (1853-1938), who developed the technique in 1884 (Gram 1884). The importance of this determination to correct identification of bacteria cannot be overstated as all phenotypic methods begin with this assay.

The Basic Method

1. First, a loopful of a pure culture is smeared on a slide and allowed to air dry. The culture can come from a thick suspension of a liquid culture or a pure colony from a plate suspended in water on the microscope slide. Important considerations:
    - Take a small inoculum – don't make a thick smear that cannot be completely decolorized. This could make gram-negative organisms appear to be gram-positive or gram-variable.
    - Take a fresh culture – old cultures stain erratically.
2. Fix the cells to the slide by heat or by exposure to methanol. Heat fix the slide by passing it (cell side up) through a flame to warm the glass. Do not let the glass become hot to the touch.
3. Crystal violet (a basic dye) is then added by covering the heat-fixed cells with a prepared solution. Allow to stain for approximately 1 minute.
4. Briefly rinse the slide with water. The heat-fixed cells should look purple at this stage.
5. Add iodine (Gram's iodine) solution (1% iodine, 2% potassium iodide in water) for 1 minute. This acts as a mordant and fixes the dye, making it more difficult to decolorize and reducing some of the variability of the test.
6. Briefly rinse with water.
7. Decolorize the sample by applying 95% ethanol or a mixture of acetone and alcohol. This can be done in a steady stream, or a series of washes. The important aspect is to ensure that all the color has come out that will do so easily. This step washes away unbound crystal violet, leaving Gram-positive organisms stained purple with Gram-negative organisms colorless. The decolorization of the cells is the most "operator-dependent" step of the process and the one that is most likely to be performed incorrectly.
8. Rinse with water to stop decolorization.
9. Rinse the slide with a counterstain (safranin or carbol fuchsin) which stains all cells red. The counterstain stains both gram-negative and gram-positive cells. However, the purple gram-positive color is not altered by the presence of the counter-stain, it's effect is only seen in the previously colorless gram-negative cells which now appear pink/red.
10. Blot gently and allow the slide to dry. Do not smear.

What's Going On?

Bacteria have a cell wall made up of peptidoglycan. This cell wall provides rigidity to the cell, and protection from osmotic lysis in dilute solutions. Gram-positive bacteria have a thick mesh-like cell wall, gram-negative bacteria have a thin cell wall and an outer phospholipid bilayer membrane. The crystal violet stain is small enough to penetrate through the matrix of the cell wall of both types of cells, but the iodine-dye complex exits only with difficulty (Davies *et al*. 1983)



The decolorizing mixture dehydrates cell wall, and serves as a solvent to rinse out the dye-iodine complex. In Gram-negative bacteria it also dissolves the outer membrane of the gram-negative cell wall aiding in the release of the dye. It is the thickness of the cell wall that characterizes the response of the cells to the staining procedure. In addition to the clearly gram-positive and gram-negative, there are many species that are "gram-variable" with intermediate cell wall structure (Beveridge and Graham 1991). As noted above, the decolorization step is critical to the success of the procedure.

Gram's method involves staining the sample cells dark blue, decolorizing those cells with a thin cell wall by rinsing the sample, then counterstaining with a red dye. The cells with a thick cell wall appear blue (gram positive) as crystal violet is retained within the cells, and so the red dye cannot be seen. Those cells with a thin cell wall, and therefore decolorized, appear red (gram negative).

It is a prudent practice to always include a positive and negative control on the staining procedure to confirm the accuracy of the results (Murray et al 1994) and to perform proficiency testing on the ability of the technicians to correctly interpret the stains (Andserson, et al. 2005).

Excessive Decolorization

It is clear that the decolorization step is the one most likely

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to cause problems in the gram stain. The particular concerns in this step are listed below (reviewed in McClelland 2001)

- Excessive heat during fixation
  Heat fixing the cells, when done to excess, alters the cell morphology and makes the cells more easily decolorized.
- Low concentration of crystal violet
  Concentrations of crystal violet up to 2% can be used successfully, however low concentrations result in stained cells that are easily decolorized. The standard 0.3% solution is good, if decolorization does not generally exceed 10 seconds.
- Excessive washing between steps
  The crystal violet stain is susceptible to wash-out with water (but not the crystal violet-iodine complex). Do not use more than a 5 second water rinse at any stage of the procedure.
- Insufficient iodine exposure
  The amount of the mordant available is important to the formation of the crystal violet - iodine complex. The lower the concentration, the easier to decolorize (0.33% - 1% commonly used). Also, QC of the reagent is important as exposure to air and elevated temperatures hasten the loss of Gram's iodine from solution. A closed bottle (0.33% starting concentration) at room temperature will lose >50% of available iodine in 30 days, an open bottle >90%. Loss of 60% iodine results in erratic results.
- Prolonged decolorization
  95% ethanol decolorizes more slowly, and may be recommended for inexperienced technicians while experienced workers can use the acetone-alcohol mix. Skill is needed to gauge when decolorization is complete.
- Excessive counterstaining
  As the counterstain is also a basic dye, it is possible to replace the crystal violet—iodine complex in gram- positive cells with an over-exposure to the counterstain. The counterstain should not be left on the slide for more than 30 seconds.

Alternatives to the Gram Stain

Gram's staining method is plainly not without its problems. It is messy, complicated, and prone to operator error. The method also requires a large number of cells (although a membrane-filtration technique has been reported; Romero, et al 1988). However, it is also central to phenotypic microbial identification techniques.

This method, and it's liabilities, are of immediate interest to those involved in environmental monitoring programs as one of the most common isolates in an EM program, *Bacillus* spp., will frequently stain gram variable or gram negative despite being a gram-positive rod (this is especially true with older cultures). The problems with Gram's method have lead to a search for other tests that correlate with the cell wall structure of the gram-positive and the gram-negative cells. Several improvements/ alternatives to the classical gram stain have appeared in the literature.

KOH String Test

The KOH String Test is done using a drop of 3% potassium hydroxide on a glass slide. A visible loopful of cells from a single, well-isolated colony is mixed into the drop. If the mixture becomes viscous within 60 seconds of mixing (KOH-positive) then the colony is considered gram-negative. The reaction depends on the lysis of the gram-negative cell in the dilute alkali solution releasing cellular DNA to turn the suspension viscous. This method has been shown effective for food microorganisms (Powers 1995), and for *Bacillus* spp (Carlone et al 1983, Gregersen 1978), although it may be problematic for some anaerobes (Carlone et al 1983, but also see Halebian et al 1981).

This test has the advantage of simplicity, and it can be performed on older cultures. False negative results can occur in the test by using too little inoculum or too much KOH (DNA-induced viscosity not noticeable). False positive results can occur from too heavy an inoculum (the solution will appear to gel, but not string), or inoculation with mucoid colonies. This can serve as a valuable adjunct to the tradition gram stain method (von Graevenitz and Bucher 1983).

Aminopeptidase Test

L-alanine aminopeptidase is an enzyme localized in the bacterial cell wall which cleaves the amino acid L-alanine from various peptides. Significant activity is found almost only in Gram-negative microorganisms, all Gram-positive or Gram-variable microorganisms so far studied display no or very weak activity (Cerny 1976, Carlone et al. 1983). To perform the test, the reagent is used to make a suspension (with the bacteria). Aminopeptidase activity of the bacteria causes the release of 4-nitroaniline from the reagent, turning the suspension yellow. The test is especially useful for non-fermenters and gram-variable organisms, and is a one step test with several suppliers of kits. Results of the test are available in 5 minutes.

Fluorescent Stains

A popular combination of fluorescent stains for use in gram staining (particularly for flow-cytometry) involves the use of the fluorescent nucleic acid binding dyes hexidium iodide (HI) and SYTO 13. HI penetrates gram-positive but not gram-negative organisms, but SYTO 13 penetrates both. When the dyes were used together in a single step, gram-negative organisms are green fluorescent by SYTO 13 while gram-positive organisms are red-orange fluorescent by HI which overpowers the green of SYTO 13 (Mason *et al* 1998). There are commercial kits available for this procedure, which requires a fluorescent microscope

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USP 29/NF 24. 2006. Chapter <1231> Water for Pharmaceutical Purposes. United States Pharmacopeial Convention.

Future articles *will deal with the knotty issues of how to establish meaningful Alert and Action Levels for your water systems, how to select an optimal microbial test method, how to handle testing and possible alert/action levels and specifications for source water, and other worrisome issues that make your on-call pager go off in the middle of the night.*

About the Author

Dr. T. C. Soli, Principal Consultant and President of Soli Pharma Solutions, Inc. (SPS), is a Ph.D. microbiologist who has acquired considerable knowledge of pharmaceutical water systems through his 26 years of pharmaceutical industy experience with 5 pharmaceutical operating companies and consulting with career-long memberships on numerous industry-wide working committees related to water, including his current Vice Chairmanship of the USP Pharmaceutical Water Expert Committee. He can be reached by phone at (252) 902-5097 or (252) 795-4779. Email: solipharmasol@earthlink.net Website: www.solipharmasol.com.

USP Corner

Revalidation of Microbial Limits Tests?

The Microbial Limits chapters were finalized by the PDG (Pharmacopeial Discussion Group, made up of representatives of the JP, EP, and USP) in November of 2005. The pharmacopeia have not come to a publication schedule, but the finalized chapters are not reported to be significantly different from those published for comment in 2003:

USP. 2003. <61> Microbiological Examination Of Nonsterile Products: Microbial Enumeration Tests. *Pharm Forum*. 29(5):1714-1722.

USP. 2003. <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms. .*Pharm Forum*. 29(5):1722-1733.

USP. 2003. <1111> Microbiological Quality of Nonsterile Pharmaceutical Products. *Pharm Forum*. 29(5):1733-1735.

Next month the *PMF Newsletter* publish a review of the microbial limits tests **with an eye towards the need to revalidate product tests**. This review will compare the draft documents (above) against the current testing requirements of the USP and the Pharm Eur.

Any questions concerning USP documents should be sent to Radhakrishna (Radha) Tirumalai, Ph.D. You can reach Dr. Tirumalai at: (301) 816-8339, via mail at United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790 or via e-mail at **RST@USP.org**. You can write representing your company, or as an individual scientist

Internet Address	Description
http://www.cfsan.fda.gov/~ebam/bam-toc.html	FDA's Bacterial Analytical Manual (BAM) is online. Excellent source of procedures, also two interesting appendices providing reviews of Rapid Methods (Food) and the Most Probable Number (MPN) method.
http://www.microbelibrary.org/	ASM's (American Society for Microbiology) collection of training materials and images for microbiologists.
If you have found an Internet site that contains information of relevance to pharmaceutical microbiology, please let us know.	

Upcoming Events

February

- 15th – 16th **PMF's Bacterial Endotoxin Summit** hosted by Karen McCullough
  Location: Doubletree Hotel Philadelphia
  Phone: 888-844-8561 (toll-free, North America)
  or +1 585-594-3336
  Email: register@highpeaks.us
  Web Site: http://www.highpeaks.us/2006/BES/

- 16th – 17th **Investigating Microbial Contaminations** Course
  Location: Crowne Plaza (Foster City, San Francisco Area)
  Phone: +1 408-445-0507
  Web Site: http://www.microrite.com/Investigating_Microbial_Contaminations_San_Francisco_February_2006.pdf

March

- 13th -14th **Species and speciation in micro-organisms**
  Location: The Royal Society, 6-9 Carlton House Terrace, London SW1Y 5AG.
  Phone: 020 7451 2500
  Email: events@royalsoc.ac.uk
  Web Site: www.royalsoc.ac.uk/events

- 13th – 15th **Microbiology Course Series**
  - Validation of Microbiological Methods
  - Auditing QC Microbiology Laboratories
  - Water System Microbiology
  - Microbial Identification Methods

  Location: San Francisco Bay Area
  Phone: 888-844-8561 (toll-free, North America)
  or +1 585-594-3336
  Email: register@highpeaks.us
  Web Site: http://www.highpeaks.us/upcoming.htm

- 19th – 22nd **Annual Conference of the Association for General and Applied Microbiology**
  Location: Friedrich-Schiller-University Jena, Germany
  Phone: +49(0)3641 35 33 15
  Email: vaam@conventus.de
  Web Site: http://www.vaam.de; http://www.conventus.de/vaam

- 23rd – 24th **Investigating Microbial Contaminations** Course
  Location: Caribe Hilton (San Juan, Puerto Rico) Phone: +1 408-445-0507
  Web Site: http://www.microrite.com/Investigating_Microbial_Contaminations_Puerto_Rico_March_2006.pdf

- 27th - 28th **2006 International Conference on Biocontainment Facilities**
  Location: St. Petersburg, FL
  Phone: 925-254-1744
  Email: bill@tradelineinc.com
  Web Site: http://www.tradelineinc.com/bio

- 27th - March 1 **Managing Risk in Aseptic Filling and Processing for Pharmaceutical and Biopharmaceutical Products**
  Location: London
  Website: http://www.iir-events.com/IIR-Conf/page.aspx?id=246

- 29th - 31st **The Environmental Monitoring and The Stability Technical Seminar and Workshop**
  Location: Hilton New York, NY
  Phone: 514-788-6023
  Web Site: http://www.novaseminars.com

Offering In-House Courses on Microbiology/ Aseptic Processing:

- The Microbiology Network/High Peaks Associates http://www.highpeaks.us/in-house.htm

- USP
  Contact Steven Paul (stp@usp.org) for information on the course "Fundamentals of Microbiological Testing"

Discussion List Update

PMFList:
Number of Subscribers: 1,400
Number of Countries: 49
Number of Messages Last Month: 208
PSDGList:
Number of Subscribers: 759
Number of Countries: 19

Are you aware of our on-line discussion group?

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

You can ask, answer, or read questions and comments from your colleagues.

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or a flow cytometer.

Sizemore *et al* (1990) developed a different approach to fluorescent labeling of cells. Fluorescence-labeled wheat germ agglutinin binds specifically to N-acetylglucosamine in the outer peptidoglycan layer of gram-positive bacteria. The peptidoglycan layer of gram-negative bacteria is covered by a membrane and is not labeled by the lectin. A variant of this method has also been used to "gram stain" microorganisms in milk for direct measurement by flow cytometry.

LAL-based Assay

Charles River Laboratories has just released a product to be used with their PTS instrument – the PTS Gram ID (Farmer 2005). This methodology makes use of the same reaction used for the chromogenic LAL test. Gram-negative organisms, with bacterial endotoxin, initiate the LAL coagulase cascade which results in activation of the proclotting enzyme, a protease. In the LAL test, this enzyme cleaves a peptide from the horseshoe crab coagulen, resulting in a clot. It can also cleave a peptide from a synthetic substrate, yielding a chromophore (p-nitroaniline) which is yellow and can be measured photometrically at 385 nm (Iwanaga 1987). Gram-positive organisms, lacking endotoxin, do not trigger the color change in this method, while gram-negative organisms do trigger it. Results are available within 10 minutes.

Summary

The differentiation of bacteria into either the gram-positive or the gram-negative group is fundamental to most bacterial identification systems. This task is usually accomplished through the use of Gram's Staining Method. Unfortunately, the gram stain methodology is complex and prone to error. This operator-dependence can be addressed by attention to detail, and by the use of controls on the test. Additional steps might include confirmatory tests, of which several examples were given. As with all microbiology assays, full technician training and competent review of the data are critical quality control steps for good laboratory results.

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About the Author

Dr. Sutton is Pharma Consultant (Microbiology) with Vectech Pharmaceutical Consultants, Inc. There he has assisted companies as varied as start-ups, generics, and established pharmaceutical innovators with laboratory management and compliance issues, process change opportunities, technical research, project management, and implementation of rapid microbiological methodologies. Dr. Sutton is an active author and speaker with over 30 publications in print. He is also active in a volunteer capacity, being involved with the USP Analytical Microbiology Committee of Experts since 1993, and in serving as a reviewer for several journals as well as moderating the PMFList and the PSDGList (http://www.microbiol.org). He can be contacted at scott.sutton@microbiol.org, or at +1 (585) 298-0767.

Mike Korczynski, a Gentleman, a Scholar, and a Friend

It is indeed a sad day to have to announce that Michael Korczyski, PhD has passed away last week in Bolivia, NC. The flurry of E-mails I received from people associated with Mike through the years and the sentiments expressed in these messages indicated that he was respected by all as an individual, as a scholar, and as a friend.

Mike K. as he was called by most with affection, or because they could not spell his name correctly, was respected as an individual because of his honesty, always ready to help his fellow scientists, a very hard worker, yet a gentle person. I have known Mike for over 35 years and have never heard criticisms of Mike the individual from any quarter. Few of us, if any, can have that record! Those who have crossed paths with Mike would say the same and he will be remembered fondly by all.

Mike's contributions to the science of microbiology and sterilization are numerous and seminal, perhaps too numerous to recount here. As one of his contemporaries wrote, he was able to convey difficult subjects in simple words that everyone could understand.

Our paths crossed several times and I was richer because of it each time. Early, we both worked at Abbott Laboratories. We were in different divisions, but had microbiology in common. Later he joined USP as a member of the Microbiology Subcommittee in the late 1980's and remained a member until 2005. Dr. Joseph Knapp, former Chair of the USP Expert Committee indicated that "I could always count on Mike to take on jobs and bring them to fruition with a solid result". As a liaison for that Expert Committee early in Mike's tenure I can vouch for that statement. Mike's contributions to the development of standards for sterilization, biological indicators, and microbiological testing were always on target, even when not the "common wisdom" of the time. His theoretical understanding of microbiology principles was followed by a practical application of these principles and as such enhanced the value of microbiology in industrial settings. Even after he retired from the USP Expert Committee on microbiology he was always ready to help if called upon.

Our paths crossed again since we were both involved in PDA in various leadership positions. Mike was first elected as a Director of the PDA Board of Directors, then was elected by the membership as President of PDA, which is another tribute to his standing in the industry. Following his retirement from Abbott Laboratories, he joined PDA staff as Vice President of Education and Director of the Training & Research Institute at the University of Maryland, Baltimore County. Against the "common wisdom" of the time he made the Training Institute a functioning entity servicing the needs of industry and regulatory agencies for training in a variety of areas. However, the hallmark of his tenure was the development of a hands-on, two-week training course for aseptic processing. This course is so successful that it is always oversubscribed because of a need that Mike was able to envision.

Good microbiologists never retire, they become consultants. Mike founded a consulting group MIKKOR that became very successful. However, he divided his time between his consultant work and his love for the sea (sailing) and the turf (golf) by spending six months in Bolivia, NC and six months in his home in the suburb of Chicago, IL.

I am sure that I speak for all the people that have crossed Mike's path in presenting our sincere condolences to his beloved wife and children. This is really a sad day for all of us and for microbiologists, but Mike K's memory and legacy will always live in the minds and hearts of all. I am privileged to have had Mike as a friend.

Roger Dabbah, PhD
USP

Note from the editor:

At the request of Dr. Korczynski's family, anyone who wishes to may make a donation "In Memory of Michael S. Korczynski" to:

Syracuse University
Advancement Office
307 Hall of Languages
Syracuse, NY 13244

Donations will support the university's summer biology intern program.