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The Only Constant is Change

Scott Sutton, Ph.D
Editor, PMF Newsletter

2005 was a year of rebuilding for the PMF Newsletter. Any publication of this type depends on the interest and contributions of it's participants for content and support. We know that interest is high in QC and regulatory microbiology issues due to the frequent and animated discussions on the Email list, the PMFList. However, after many years of publication, the *PMF Newsletter* was not published for most of 2004 and all of 2005 as the PMF underwent a change. One of the biggest aspects of that change involves the operation and purpose of the PMF.

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

The PMF has moved away from a Traditional "User's Group" where the members paid dues on an annual basis. The support of PMF now comes from sponsored conferences, and industry support of this newsletter. The newsletter is also changed - no longer available in print, but Emailed to all concerned. This expands and facilitates distribution of the newsletter to an extensive audience.

Interested authors have two avenues for publication. Standard articles are actively solicited and encouraged for rapid publication. These articles can be devoted to topics of opinion/regulatory issues, laboratory procedure aids, preliminary publication of data or other topics of interest to the practicing microbiologist. A second avenue for publication is an occasional column from a representative of the organization "Microbiologists for Common Sense and Reason" (MCSR - thanks to George Spite for the concept) which will examine more controversial topics without author attribution. These MCSR articles are designed to strenuously discuss current practice from a purely scientific perspective. Membership in MCSR is open. Both article types will be held to high standards of data and/or scientific literature support.

I appreciate your continued interest in the PMF Newsletter, the PMFList and the Stability Discussion Group Email List (PSDGList) and I am certain that with your support, we will have a great and successful 2006.

Quality Control of Microbiological Culture Media

Scott Sutton, Ph.D
Vectech Pharmaceutical Consultants

Quality control of microbiological culture media is central to the success of the QC microbiology laboratory (USP 2004). This is reflected by recent changes in the pharmacopeia, both implemented and proposed, have increased the importance of media growth promotion (GP) studies to compendial testing (Cundell 2002, Sutton, 2005). The harmonized Sterility Test (USP 2003a) incorporates requirements for regular sterility testing of media, and the PIC/S recommendation extends this expectation to growth promotion testing of spent media (the "Stasis Test" – see PIC/S 2002). The recently harmonized Microbial Limits Tests (USP 2003b, USP 2003c) expand the requirements to an evaluation of the differential and selective properties of the media, in addition to confirming the nutritive properties. Finally, the proposed USP chapter on microbiological laboratory practices stresses the need to adequately control the growth media (USP 2004). None of these documents, however, provides detailed information on how to establish the overall quality attributes of media.

Although Growth Promotion Testing is the most obvious example of media quality control measures, it is by no means the only measure that a QC microbiology laboratory should employ. In this article we will group the methods used to maintain the quality of microbiological media in four headings:

- Control of Preparation
- Testing of Physical and Chemical Parameters
- Growth Promotion Testing
- Control of storage conditions

Control of Media Preparation

QC laboratories acquire media in one of two ways, either purchasing the media pre-made from a manufacturer, or making the media (either in whole or in part) in-house. These preparation schemes must be considered separately.

Clearly, if the media is purchased from the vendor there is little opportunity to control the preparation beyond having confidence in the supplier. However, agar acquired in large aliquots for pour-plates must be carefully melted prior to use – this melting must be under controlled conditions to avoid damaging the media. Of course, all media used is expected to be checked for physical and chemical parameters and growth promotion (see below), and prepared media is no exception to this expectation.

Media prepared in-house offers several opportunities for quality control. The raw materials (either the dehydrated complete media or the components) must be stored under appropriate and controlled conditions and used within established expiry dates. The compounding of the media must be controlled to ensure the media is prepared correctly. Agar media must be pre-warmed to dissolve the agar prior to sterilization, but not heated so extensively as to damage any heat-labile components. The sterilization procedure also must be under control. Normally this means using a validated autoclave cycle (and load configuration) shown to hold the media at 121^{o}C for 15 minutes (note that this is not the same condition as a 15 minute cycle with a maximum temperature of 121^{o}C). Each batch of media should be clearly labeled to allow for unambiguous audit of each stage of preparation.

Control of Physical and Chemical Parameters

The goal of this testing is to provide a gate-keeping function before investing the time in growth-promotion testing. pH of the finished media (pH measurement must be conducted at room temperature unless specific allowance is made for the temperature) is a critical attribute to confirm. The color of the media should be examined and a decision made as to its correctness, as well as an examination for any crystal formations or variations in color (for agars). The containers of media should be thoroughly examined for cracks or defects, and all defective units discarded. There are additional checks that can be performed (HPLC of major components, determination of sugar concentration, *etc.* Curtis 1985), but these are not normally conducted in the pharmaceutical QC lab.

Growth Promotion Testing

There are some significant concerns as to the need for GP testing of standard media. It can be argued that since all preparation conditions are under control and the physical parameters of the finished media is checked, there is little additional information gathered by the labor-intensive and time-consuming procedure of checking the growth promoting capabilities of the media. This topic has been debated not only among workers in QC laboratories, but also in the clinical microbiological industry.

Clinical microbiology laboratories in the United States

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are not required to test most common media under NCCLS standard M22-A2 "Quality Assurance for Commercially Prepared Microbiological Culture Media" although this stance has come under question by the relevant NCCLS committee and is being re-evaluated (Krishner 1999). The current understanding of which clinical media to test was based on a survey performed in the early 1980's of 1,164 laboratories. From their reported experiences it was determined that most media could be accepted safely on the manufacturer's data (Krishner 1999). This result confirmed an earlier study (Nagel and Kunz 1973) that called into question the need for excessive growth-promotion testing of commercially prepared media. They examined 900 lots of 46 different media representing 350,000 units of purchased culture media, and found only 17 lots to be unsatisfactory. These lots were of specialized media containing labile components.

There has been no convincing scientific evidence published that would argue for the need to test Trypticase Soy media, for example, for growth promotion. However, both the Sterility Test and the Microbial Limits Tests require such testing. Given the compendial requirement to test, the first decision may reasonably be to determine the challenge organism. In addition to the compendial organisms required in the tests, addition of specific microorganisms of interest could be useful if they have been recovered from past tests (*e.g.* a Sterility Test contaminant or a frequent environmental monitoring isolate).

The next concern is test design. There are two types of media commonly used in the microbiological lab – broth and agar. These two types must be considered separately as they show growth by completely different means. The fundamental question of GP testing can be expressed as: Is the new batch of media as good as a previously qualified batch? This question cannot be answered adequately except by statistical comparison, given the variability of microbio-logical data. The statistical design of GP studies will be developed in the following discussion which has been influenced by the excellent review by Weenk (1992).

Growth Promotion Testing of Agar Media

A singular advantage of agar media tests is that they provide numbers – colony forming units (CFU). To analyze CFU you must use statistical tools designed for the Poisson distribution (Ilstrup 1990) or else convert the data to approximate the normal distribution. This data conversion can be done by using its $\log_{10}$ values or by taking the square root of (n+1) (Ilstrup 1990). Once this is done, plate counts can be directly compared using "Student's" T Test or other tests of normally distributed data.

There are, of course, several less demanding tests for demonstration of equivalency between two agars:

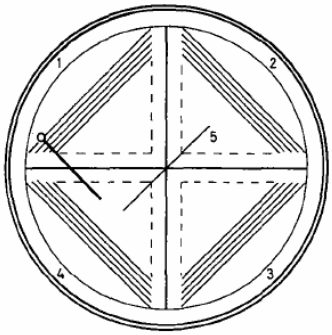
Spread Plates or Pour Plates

The compendia assume a GP test by comparison of CFU, with the cells plated in the normal fashion for the lab. The compendia generally require that the colony counts derived from growth on the current batch of media be no less than 50% (USP 2003b) or 70% (USP 2004) of a previously qualified batch. This approach provides the advantages of colony counts and a large area for the colonies to grow, but it is somewhat laborious and expensive in terms of material.

Miles-Misra (Drop Count) Technique

This technique involves dropping the cells in a 10 µL aliquot onto the surface of an agar plate (Miles and Misra 1938). When used carefully, an entire 6-fold dilution scheme can be plated in a single Petri dish and if read early, the individual drops can be used to yield estimates of the number of CFU/mL in the challenge suspension. This method offers significant advantages in terms of labor and material resources.

Ecometric Method



This method is a variation of streaking to extinction. A fresh suspension of the challenge organism is taken into a calibrated loop and streaked in five parallel lines over four sections of an agar plate in sequence, then once through the middle (image from Mossel 1980). These plates are then incubated overnight for growth. The patterns of growth are interpreted to provide an Absolute Growth Index (AGI):

Growth	AGI
All Streaks	5.0
All but middle streak	4.0
All in quadrants 1, 2, and 3 but half in quadrant 4 and none in middle streak	3.5
All in quadrants 1, 2, and 3 but no growth in quadrant 4 or middle streak	3.0
Growth scored on half quadrant scores to - 2.5, 2.0, 1.5 and so on.	

This technique is somewhat operator-dependent and offers a lower precision than those yielding CFU, but can

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be used to great effect with practice (Mossel 1980).

Growth Promotion Testing of Broth Media

Copious Growth

This is the current compendial method of choice. In this method, the challenge organism is inoculated at a very low level (< 100 CFU per unit) and incubated at the prescribed temperature for the prescribed period of time (3 days or 5 days). Growth in the batch of media is then compared to a parallel sample from a previously qualified batch of the same media. The growth is to be comparable between the two and copious. The advantage of this method is that it does not require a great deal of labor, but the quality of the data for the comparison between the growth promoting characteristics of the media is exceptionally poor. This can be described as a crude end-point test with an "n" of 1.

End-point Methods

In this approach to growth promotion testing, very low levels of inoculum are added to multiple tubes of the two media being examined. Then the resultant growth frequency is compared between the two media to determine equivalency. For example, comparing an old and a new batch of Trypticase Soy Broth (Soy Bean Casein Digest Broth) might be performed by taking 100 tubes of each media, and then inoculating all 200 tubes with <5 CFU of the challenge organism *Staphylococcus aureus*. After incubation, the number of turbid tubes would be compared – say 30/100 of the new media turbid vs. 46/100 of the old media. The statistical comparison could be performed using the Chi Square Test or Fisher's Exact Test. This evaluation would be performed separately for each challenge organism. The number of tubes used can be decreased (or increased) at the expense of the statistical power of the method.

End-point methods to growth promotion of broth media are obviously very laborious and technically demanding. It is not difficult to envision a design that would require more than a thousand tubes and the need to accurately create an inoculum of <5 CFU of a variety of challenge microorganisms.

MPN

The Most Probable Number method of enumerating microorganisms is most commonly used in the QC lab as part of the Microbial Limits Test (USP 2003b) or in other situations where the sample cannot be put into an appropriate suspension or be filtered (Aspinall and Kilsby 1979). In this technique, the unknown sample is prepared in a ten-fold dilution series and added to nutrient broth in replicate tubes (normally either 3, 5 or 10 replicates are used). The tubes will then either turn turbid (growth) or remain clear, and allow for an estimate of the most probable number of microorganisms. The question being asked in this experimental design is "At what point does the unknown number of organisms become so dilute as to fail to inoculate the growth media?"

A complete discussion of this technique may be found on the FDA web site as the second appendix to the online version of the Bacteriological Analytical Manual (http://www.cfsan.fda.gov/~ebam/bam-a2.html). The tables included in this appendix also provide 95% confidence intervals for the estimates of the most probable number.

To use this technique for growth promotion testing you must start with a known concentration of microorganisms and then ask the question "Do my two media provide the same estimate of the most probable number of CFU from identical inocula?" This is best done by using a low inoculum (approx 50 CFU in the first dilution). The inoculum is serially diluted (ten-fold), and added to the two broths in a 3-tube or a 5-tube design. After incubation, the MPN of the two media are determined (remember, starting from the same inoculum). If the new media is to be qualified, it should not yield an MPN with a confidence interval that is below the lower limit of the confidence interval of the previously qualified batch.

This technique for growth promotion testing of broths offers the advantages of being much less expensive in terms of time and resources than the other broth techniques (with the exception of the compendial tests) as well as being very forgiving about the concentration of the starting inoculum. It is by far the easiest method to provide a statistical comparison between the growth promoting capabilities of two broth media batches. This approach can be made much easier as well by using commercially available starting inocula of defined numbers (such as BTF's BioBall - Morgan 2004).

Kinetic Parameters

The growth promoting capabilities of two batches of broth can be compared by measuring the growth curves of identical inocula grown side-by-side. The growth rate of the challenge organism in the broth can be determined either spectrophotometrically or by viable count to provide a sensitive means to compare the nutritive properties of the media. However, this method is extremely labor intensive. A second method using kinetic parameters is to compare

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the length of the lag phases of the same inoculum on the two media. The comparison of lag phase measurements suffers the same disadvantage of labor usage, and can be very difficult to implement and subject to significant variability. Neither of these methods is practical for the QC microbiology lab due to their high labor requirements.

GP Summary

The best overall design for GP studies of agar media would be through the Miles-Misra technique as it is economical (both in material and labor), and provides colony counts. The best design for GP studies of broth media is the MPN design which allows statistical comparison between the media batches without requiring large investments of time and material.

Control of storage conditions

Media Quarantine and Release

The laboratory must have some procedures in place to prevent unqualified media from entering the testing process. This ideally would be a separate storage room from that used to store qualified media, but may also be accomplished through tagging the quarantined material and placing it in a clearly identified area within the same room. All quality control checks on the quarantined media should be completed before its documented release for general use. Storage conditions of the quarantined media should match those of the released media.

Media Storage and Expiry

Media should always be stored under controlled conditions to ensure its quality through to the expiry date. Factors to be evaluated in these controlled conditions include:

- Temperature
- Container (glass, plastic, container closure system, etc)
- Humidity
- Light

Although all these factors may not be a concern for all media, they can be a concern for different types. For example, Trypticase Soy Agar is robust and can tolerate a wide range of storage conditions (assuming appropriate temperature control) though the stability period. However, Dey-Engley Broth (Dey Engley 1983), a broth commonly used to neutralize a variety of disinfectants and preservatives, will degrade upon exposure to oxygen and so must be stored in oxygen-impermeable material in well-sealed container. Similarly, Fluid Thioglycollate Medium needs to maintain a highly reduced state for recovery of anaerobes and so must have oxygen excluded. This medium, used in the Sterility Test (USP 2003a) incorporates resazurin as a redox indicator which turns the medium pink if exposed to oxygen. The Sterility Test procedure calls for action if the upper third of the media is pink in color.

The expiry date of media may be set by the vendor of purchased media, but must be established by the lab for in-house media. This dating can draw upon the compendia for guidance. The Sterility Test states that:

> "If prepared media are stored in unsealed containers, they can be used for 1 month, provided that they are tested for growth promotion within *2* weeks of the time of use and that color indicator requirements are met. If stored in tight containers, the media

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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

Internet Address	Description
http://scholar.google.com/	Google's extraordinary link to scholarly literature available on the web. Entering search terms in the familiar Google search box returns articles available as HTML or PDF documents.
http://science.nhmccd.edu/biol/animatio.htm#micro	An interesting collection of microbiologically-related animations.
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
  - Identification of Fungi (1 day)

  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: 1,338
Number of Countries: 49
Number of Messages Last Month: 246

PSDGList (Pharma Stability Discussion Group):
Number of Subscribers: 749
Number of Countries: 19

Are you aware of our on-line discussion group?

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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.

PAT and Real Time Release – Is This Proposal Supportable?

By a representative of "Microbiologists for Common Sense and Reason" (MRSC)

There have been several articles recently published that promote PAT as the path to real-time-release (RTR) of finished product. Simply put, this argument may work for chemistry assays of finished product quality (if there), but is feasible only if the microbiology product release requirements are ignored.

Let's take a look at a couple of recent articles promoting PAT for RTR. In the first, Process Analytical Technology is described as the only path to Real Time Release:

> "The enabling process, the PAT framework, thus has two components: '(1) a set of scientific principles and tools supporting innovation and (2) a strategy for regulatory implementation that will accommodate innovation.' Its ultimate goal is control to quality through real-time control of manufacturing, thus facilitating real-time release and continuous optimistion…'Real time release' as defined by the FDA, 'is the ability to evaluate and ensure the acceptable quality of in-process and/or final product based on process data.' Typically, the PAT component of real time release includes a valid combination of assessed material attributes and process controls. Material attributes can be assessed using direct and/or indirect process analytical methods. The combined process measurements and other test data gathered during the manufacturing process serve as the basis for real time release of the final product and would demonstrate that each batch conforms to established regulatory quality attributes.' Therefore real time release can only be achieved with demonstration of process understanding, real time control and quality assurance during manufacturing." (Afnan, 2005)

As an aside, it is an interesting argument being presented here (from the PAT guidance document). RTR is defined as the ability to evaluate and ensure quality through the use of in-process data, therefore the only way to achieve RTR is through the use of in-process data to release the product in real time. Leaving behind the cyclical nature of the argument, it is leads to an unreasonable definition. Real time release of product, at least to those involved in manufacturing, means you can release the product within a few days (preferably a few hours) of manufacture and that you don't have to wait around for the microbiology results (or other release testing and batch sign-off issues) to ship finished product.

The feasibility of RTR through process control is also discussed by Clark (2005). He develops some of the issues involved in linking process controls to final product specifications, noting that concerns include unit to unit variability of the attribute and control of that variability. This same journal issue includes a separate article from an industry commentator who is very supportive of RTR through the PAT initiative (Szechnski, 2005). Unfortunately this article, and the arguments, dealt exclusively with the chemistry controls of the process and of the finished product.

Webber (2005) provides a more comprehensive evaluation of RTR within PAT:

> "According to ICH Q6B [8], specifications are critical quality standards that establish the set of criteria to which a drug substance, drug product, or materials at other stages of its manufacture should conform to be considered acceptable for its intended use. It is noteworthy that this document does not say that the final product *must* be tested to demonstrate its conformance to specifications, simply that it *will* conform to specifications when it is tested. Therefore, while specifications are integral to describing the design space of the product, how a manufacturer assures that their product conforms to its specifications remains flexible. Specifications must be established and included in a marketing application, but product quality

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> can be ensured by methods other than lot release testing. Process analytical technology is just such a method and although it may not be possible to eliminate all lot release testing at this time, PAT holds the promise to minimize the number of tests that need to be done at release."

This description notes that PAT may be able to minimize the number of tests, but not necessarily eliminate all finished product testing. Unfortunately, the product release tests most likely to remain include the microbiology assays, which in large part determine the product release quarantine period (Singer and Cundell 2003).

The enthusiasm for PAT and RTR is not restricted to North America. A recent article by a British regulator displays the cautious desire to apply process controls exclusively to justify immediate product release (Graffner, 2005):

> "By the combination of high product and process knowledge and the introduction of new technologies in manufacturing, the possibility to apply RTR may be a process specification instead of release following testing of the finished product. . . How the manufacturer ensures RTR-compliance with the release specification is thus open to discussion but it has to be described in the submission… Such a specification has to recognize the interrelationship between various process parameters and describe ranges within which the process needs to be operated.
>
> Independent of process specification is the relationship between process measurements and controls and release specifications."

In this passage we gain some insight into the regulatory thinking. Firstly, the manufacturer must determine how measuring physical parameters will ensure product sterility (or bioburden and absence of objectionable microorganisms for non-sterile product release) and secondly the manufacturer must also determine their ranges – *e.g.* demonstrate at what point the product becomes unacceptably contaminated based on these in-process parameters. This places the entire burden of providing the justification of real-time-release based on process parameters on the manufacturer.

RTR through PAT requires extensive understanding and control of the process. Current in-process monitoring capabilities in microbiology do not allow for determination of finished product attributes (Farrington 2005), and in fact the accuracy of the data developed by microbiological means for in-process monitoring is open to significant question (Hussong and Madsen, 2004). One exciting approach, at least for aseptically produced products, is to encase the entire manufacturing process in a barrier isolator system (Agalloco and Akers 2005), but even there we are only producing a high aseptic environment, not a sterile one. Therefore we are left without any reliable data on the microbiology of the process or its effects on finished product quality. Without that assurance, the microbiological considerations prevent RTR for any product (sterile or non-sterile) strictly through process controls.

There have been attempts to incorporate microbiology into the discussion of Process Analytical Technology. The first approvals under the PAT initiative were for rapid microbiological release tests (Newby, *et al*. 2004), in apparent contradiction to the current understanding of PAT. This did, however, allow the manufacturer (GSK) an opportunity to release a non-sterile product using ATP bioluminescence rather than traditional bioburden testing) for potential real time release of product.

Korczynski developed an integrated approach to product quality in a well reasoned and comprehensive review (Korczynski 2004). He argued that PAT is one of several quality initiatives (including HACCP, concurrent validation and parametric release) with the potential to dramatically improve the final product quality through control of the process. This approach could result in greatly reducing the number of number of release tests, although it could not remove the need for sterility

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testing at the present time.

Why can't we get around finished product testing for microbiology? The FDA's Aseptic Processing Guidance (FDA 2004) contains an entire section on Sterility Testing, as an example, citing multiple references in CFR that demonstrate the need for adequate testing of finished product. Similar arguments can be made for non-sterile products and the need to assay bioburden and the absence of objectionable microorganisms also a favorite topic of the Agency).

The crux of the concern, then, is that the PAT initiative is being suggested as a route to real-time-release of finished product. The concern from this representative of MCSR is that there are no adequate process controls to demonstrate product sterility or indeed any finished product microbial attributes. Therefore RTR cannot occur through PAT alone, but only in concert with rapid microbiological tests for product release.

Summary

We cannot avoid the need to demonstrate microbiological quality of the finished product, and we do not have the tools to meet this obligation through in-process controls. RTR cannot be achieved under the current regulatory climate except through parametric release of terminally sterilized products. Real time release of product cannot be achieved for aseptically produced products or for non-sterile products without a rapid test for the microbiologically-related finished product specifications.

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Book Review

Endotoxins: Pyrogens, LAL Testing and Depyrogenation 2nd Edition

Kevin L. Williams
Marcel Dekker 2001

Kevin Williams' excellent treatment of endotoxins provides a solid foundation to the understanding of bacterial endotoxins, pyrogens, and tests for bacterial endotoxin that will be of value to the QC microbiologist or the Regulatory Affairs professional. This second edition of the popular text has been expanded to include coverage of non-microbial host active compounds, as well as providing revisions in virtually every chapter.

The book is organized into 15 chapters, beginning with a review of the history of the pyrogen research, an in-depth description of the structure of endotoxins and an overview of the pyrogen-induced febrile response. Before going any more deeply into the topic of the structure of endotoxins, Dr. Williams introduces the topic of non-endotoxin pyrogenic material, both of microbial and non-microbial nature, with an impact on patient care. This is an intellectually interesting group of topics, but they may not be especially useful to the QC microbiology worker.

The QC microbiologist will find much to think about in the next chapters. These deal with manufacturing control and the use of endotoxin as a standard. Of course, no book on the subject would be complete without a discussion of the now little-used (outside of vaccine manufacture) rabbit pyrogen test, which is given due consideration.

It really isn't until the final third of the book that the LAL test is discussed in any depth, but this is due more to the extensive effort the author has placed in providing a comprehensive background than in any slighting of the coverage of the test. The history of the LAL test is thoroughly reviewed, with discussion of various applications. The gel-clot assay, the kinetic assay, and then potential new assays are examined at length. Finally, depyrogenation is presented from a theoretical perspective as well as a practical one, with useful guidance on how to design a depyrogenation validation study.

Williams closes the book with a chapter on therapeutic approaches to gram-negative sepsis contributed by N.A. Nnalue which seems a bit out of place. This chapter is largely clinical in orientation and may be of little interest to the pharmaceutical manufacturing worker.

Endotoxins: Pyrogens, LAL Testing and Depyrogenation is a comprehensive and useful treatment of the topic. The material is presented in a well organized manner, and will be of interest to both the experienced worker looking for in-depth information and the neophyte trying to get a perspective on the topic. The experienced worker will particularly appreciate the discussions on the history and development of the endotoxin tests, as well as the theoretical discussions of depyrogenation validation studies (a particularly complex topic). The chapters on non-endotoxin pyrogens may also help some OOS investigations in unusual circumstances. The neophyte will be provided a solid grounding in the importance of pharmaceutical endotoxin testing in protecting product quality and safety, as well as the need to control all pyrogenic material in the appropriate dosage forms.

A copy of this book will be provided to registrants at the PMF Bacterial Endotoxin Summit Feb. 15-16, 2006

This book shares useful features of most of the Marcel Dekker series. The table of contents demonstrates the clear organization of the text, and the extensive index provides a rapid tool to find the particular information needed. The author extensively references each chapter with current (given the 2001 publication date of the book) citations to the original literature. Overall, it is an excellent tool whether your goal is a review of the topic or an in-depth review of particular facet of endotoxins and their role in pharmaceuticals.

(Continued from page 5)

can be used for 1 year, provided that they are tested for growth promotion within 3 months of the time of use and that the color indicator requirements are met."

Finally, the proposed informational chapter "<1117> Best Microbiological Laboratory Practices" (USP 2004) devotes an entire section to media storage and can also be used to develop a defendable expiry dating policy.

Summary

We have examined four points to a quality control program for microbiological culture media:

- Control of Preparation
- Testing of Physical and Chemical Parameters
- Growth Promotion Testing
- Control of storage conditions

The importance of maintaining the quality of the media cannot be overstated – there are few things in the QC microbiology laboratory that will lead to problems with every aspects of the operation, and media is at or near the top of that very short list. Time spent ensuring culture media performance will be amply repaid in terms of data reproducibility and minimizing time spent on investigations of nonconforming results.

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