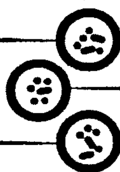

PMF NEWSLETTER

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PURPOSE: To provide a forum for discussion of microbiology issues in the pharmaceutical and related industry.
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Presidents Message

Those of you dealing with testing water per the various pharmacopeias particularly the United States Pharmacopeia (USP) and the European Pharmacopoeia (EP) have encountered that the methods for evaluation of microbial properties are different. The most striking difference resides in the medium of choice: USP recommends Plate Count Agar while EP calls for Casein Soya Bean Digest Agar. At the current time, there is no harmonization and the two methods need to be run independently to claim compliance. According to members of the Water USP Expert Committee, the USP will be calling for harmonization in the near future. We look forward to those developments and wish USP luck in being chosen the coordinating Pharmacopeia.
The joint meeting between USP and PDA is scheduled for May 19-22, 2002. The focus will be on sterile products. Hope to see some of you in Fort Myers, Florida. You can find details by visiting www.pda.org or www.usp.org.

Laura

Accuracy, Precision and Sensitivity of Microbiological Assays

By

Dr. James E. Akers
Akers Kennedy and Associates

It appears to me that one of the most common and important areas of confusion surrounding microbiological analysis has to do with the accuracy, precision and sensitivity of the methods we use to assess microbiological safety of our products and the environments in which they are made. If I were to make a generalization about the attitudes we have both within the industry, and the regulatory community, it would be that we tend to ascribe higher levels of accuracy, precision and sensitivity to microbiological assays then is scientifically warranted. I believe that there is an increasing tendency to expect more of microbiological methods than they can actually deliver. I'll give a few examples to illustrate my point and to highlight my concerns.

One of the best examples of failing to consider the sensitivity and variability of microbiological test methods has to do with active air sampling in cleanrooms. I frequently hear both, cleanroom microbiologists and individuals in the regulatory/standard setting community refer to active air sampling as "quantitative" air sampling. However, the most recent data available on the performance of various air sampling systems that we employ in our industry is that they vary significantly with respect to the results they yield. Studies indicate that these samples may vary over a range of 5-10 fold with respect to recovery rates. Reinmueller and Ljungvist did parallel controlled studies and they found that these samplers yielded very different outcomes. They found that the differences could be attributed to many things, including viable recovery rates, collection efficiency over a given particle size range and sample volume. In their study they used identical media and incubation conditions for all samplers, although industry does not have standard incubation times or temperatures, and we source media from many suppliers.

The regulatory guidelines call for critical zone airborne contamination to be < 1cfu/cubic foot or meter, however it is quite possible that none of the available methods can actually reliably measure that this "level". It is also quite probable that the results obtained may depend to a very significant degree upon the sample method chosen and the incubation time and temperature used by a firm. Therefore, both the "level" chosen as a target for air sampling in critical zones and the results obtained in sampling should, be viewed with considerable skepticism. There really is no good way to ensure that we are measuring what we believe we are measuring. As I review more and more data both from research done on air sampling and from studies done by firms in their own facilities the more convinced I am that these sampling methods are in fact semi-quantitative at best. When the accuracy, precision and sensitivity of these methods are considered it becomes clear that the use of these methods in a product release context is a scientifically dubious activity. Particularly since all of the methods are to one degree or another susceptible to inadvertent contamination during handling of samples.

A second example of unwarranted belief in precision, accuracy and sensitivity in microbiological testing is the suggestion that samples taken on critical surfaces in aseptic filling can or should be used to assess product "sterility". First, the subject of sterility is an enormously complex one. I would submit at we cannot measure the "sterility" or sterility assurance of products made in a cleanroom using microbial sampling methods. We know that swab or RODAC sampling methods are both prone to accidental contamination and quite variable in their recovery rates. Simply put there it really isn't scientifically appropriate to think that a surface sample can prove or disprove "sterility" of a surface or a product. The fact is we already know that clean rooms aren't sterile, after all, how can a room be sterile when it contains human operators? Therefore, there must be a low level of viable organisms in a cleanroom any time it is occupied regardless of how well we gown or maintain our gloves. This is an uncertainty in aseptic manufacturing that we must live with, and no amount of microbial testing will change this fact. It may be hard for us to accept that we can't prove conclusively that a product or surface is sterile, but that is our reality and we would be wise to accept it rather than try to force our tests to give us information that they simply can't be scientifically relied upon to provide.

A final example is the humble biological indicator. One of the most frequent questions that I get from microbiologists working in our industry has to do with titration of spore populations on "BIs". Quite often microbiologists will find that when they verify spore "count" on BIs they arrive at a value of $<10^6$. This is presumed to be a very serious matter since it is often, but erroneously, thought that this is something magical

(Continued on page 3)

about a population of one million or more spores on a BI.

However, let us say for example a BI was inoculated by the manufacturer so as to have a population of $1.2x\ 10^6$. Let us say that our recovery rate including plating efficiency is 90%. That means we could expect a recovery rate $1.08x\ 10^6$. This would still put us safely in the $>10^6$ range. However, that 90% mean recovery rate will vary and if the recovery rate should fall off to 80% the apparent spore concentration would be $9.6x10^5$. Of course, there are many variables that can affect apparent recovery rate. There could be, and frequently is, loss of viability of spores on the BIs over time. Serial dilutions are required and we know that there is indeterminate error associated with serial dilutions.

Given the sources of variability then, we ought not to be terribly surprised when BI population falls below 10^6. Microbiologists ought not to be subject to criticism by others in their organization when things like this happen, as they almost inevitably must. What we must understand is that in logarithmic terms, with the variability of microbial assay methods properly considered, there simply isn't a meaningful difference between $1.2x\ 10^6$ and $9x\ 10^5$! These values are microbiologically equivalent. The traditional enumeration expectation for spore populations on BIs has been -50% of label claim to +300% of label claim, which just so happens to equal +/- 0.48 log, a value you have probably seen in USP. These tolerances were chosen because it was known that these assays could be expected to have that much variability. So, the 80% recovery example I picked is well within what has been considered historically a reasonable recovery range. It would also help if we could finally disabuse our regulators and ourselves that there is something sacrosanct about a BI spore population of 10^6.

Clearly, we must not ask or expect microbial assays to be more accurate or precise than they can be. Of course, we must also not expect them to be less variable than they are, nor should we ask them to give us information about our processes that they simply can't yield. A little practical knowledge seasoned with some pragmatic common sense would make life much easier for industrial microbiologists. Frankly, a little less involvement from chemists and pharmacists in writing microbiological standards would probably help some too!

References:

1. Akers, J. 1997. Environmental monitoring and control: Proposed standards, current practices and future directions. J. Pharm. Sci. and Tech. 49(5): 204-206.
2. United States Pharmacopeia. 2001. Chapter <1116> Microbiological evaluation of clean rooms and other controlled environments, Chapter <55> Biological indicators.
3. NASA. 1971. NASA Handbook for Biological Engineers. Washington, DC.: Technical Information Services, National Aeronautics and Space Administration.
4. Reinmueller, B. 2001. Dispersion and Risk Assessment of Airborne Contaminants in Pharmaceutical Clean Rooms. Technical Bulletin No. 56. Royal Institute of Technology, Building Services Engineering Department. Stockholm, Sweden.

About the Author
Biography

JAMES E. AKERS, PH. D.

James E. Akers, Ph.D. is the President of Akers Kennedy & Associates, Inc., located in Kansas City, Missouri (phone: 816-822-7444, E-Mail: AKAIncKC@aol.com).

He has held various QA/QC management positions and served as President of the PDA from 1991 to 1993. Currently, he is a member of the USP Committee of Experts Microbiology, as well as member and chairman of various PDA Task Forces.

Fill in the blanks:

1. Lipopolysaccharide from Gram negative bacteria____________

2. Bacterial colony measurement ____________

3. Hairlike appendages associated with motion ____________

4. Frozen CO_2 ____________

5. Salt-loving organisms ____________

6. Sterility Test Media ____________

7. Heated blood agar ____________

8. Enzyme which degrades hydrogen peroxide ____________

9. Science of classification of organisms ____________

10. Use of mild heat to kill vegetative organisms ____________

continued on page 4

Dr. Akers has lectured world-wide, and taught numerous pharmaceutical technology courses world-wide including training for US/FDA. Dr. Akers has also authored several textbook chapters, edited a book on isolation technology, and authored more than 100 technical and review articles on validation, aseptic processing, contamination control, environmental monitoring and control, biotechnology, isolator technology, sterilization and disinfection, sterility testing, media fill testing, HACCP analysis, pharmaceutical microbiology and regulatory compliance.

PMF THREAD

QC of Environmental Monitoring Media

Question 1

I would like some info regarding the QC of the environmental monitoring media. The company I work with is in Phase ll for one of the most advance products. My question to you all is Do I need to QC the media used for the environmental monitoring considering the phase the company is at this time.

Reply 1

My personal belief is that you should do media QC on all your media, regardless of its use and regardless of the phase of product development you are in. I see it as basic good lab practice.

Reply 2

Yes, always QC your media. At a minimum perform growth promotion using representative strains, ATCC isolates (Bacillus species, a mold, a yeast, Gram-neg rod, Gram-pos cocci. See USP 24 <71> for recommended organisms). I would also include prevalent environmental isolates if you have them. I would include negative controls to check media sterility.

Reply 3

I would also add pH as an additional QC check.

Reply 4 In addition you cannot perform growth promotion concurrent with use for Environmental monitoring. Failure of the media to support growth, would invalidate all data collected. Without EM data, you would have to reject. Media **must** be approved prior to use for EM purposes.

Question 2

The previous question that I have made to all for advise is enclosed.

I would like to expand the question.

If you were using media already prepared by BD , which has been QC and includes C of A.

Will you still QC this media.

Reply 1

I appreciate your question because I have been in exactly the same position in a previous job. Initially, we relied solely on the certificates of analysis from our media suppliers. When the Company expanded, we added them (microbiology media suppliers) to the list for our QA team to audit. Then we finally progressed into QC tests on our media. In all that time, we had regulatory inspections and our practices were never queried. I cannot say if that was just pure chance though!!

Reply 2

Yes, because during shipping how do you know that the media was not abused by either excessive temperatures (i.e. Freezing) etc. That is why you need to QC your media. CoA are good but the FDA will want more. We follow USP 25 for QC of media.

(Continued on page 5)

Reply 3
This may be a basis for undertaking a reduced QC program, but I believe you would still need to do some basic productivity checks (may be not sterility or pH checks). It would be good practice to overlap media batches in this situation and demonstrate equivalent recovery of one or two relevant organisms.

Reply 4
As has been pointed out by others here, there is no guarantee that some friend didn't take your shipment to Tucson, AZ in August and park the truck outdoors for a week..... therefore, it's best not to just accept a C. of A. Also, I've seen a LOT of citations from FDA and others, to the effect that you have a responsibility to assure your media's efficacy in your hands.

USP Corner

The PMF recommends that you *write directly to the USP with your comments on all proposals.* You can write representing your company, or as an individual

Any questions concerning USP documents should be sent to Dr. Roger Dabbah. You can reach Dr. Dabbah at (301) 816-8336, via mail The United States Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD 20852 or via e-mail at RD @ USP. org. When communicating with Dr. Dabbah, let him know you are a PMF member.

Current Compendia

US Pharmacopeia (USP) 25 Supplement, August 1, 2002

European Pharmacopoeia (EP) 4 Supplement 4.1, 2002

Japanese Pharmacopoeia (JP) XIII 1996 / Supplement 1998

Chinese Pharmacopoeia (1995)

* If you use any other compendia, let us know for inclusion in this corner.

Warning Letters

Here are some 483's sited from Nov 2001 - Jan. 2002,

"Your firm has not performed validation of the autoclave cycles used to sterilize equipment and supplies used in the aseptic manufacture and fill of __________"

"The COA for Purified Water.... showed an aerobic plate count result of 266 cfu/mL. The purified water was used in the manufacture of __________.

The product was released.... No investigation was performed."

"Failure to have and to follow appropriate written procedures designed to prevent microbiological contamination of drug products purporting to be sterile, as required by 21 CFR 211.11 (b). For example: There was no justification available to assure that your current media and incubation temperatures are optimal for detecting flora that may be present in your facility."

"Failure to have adequately designed procedures for production and process control to assure that aseptic drug products have the identity, strength, quality, and purity they purport or are represented to possess, as required by 21 CFR 211.1. For example: Environmental monitoring limits for microbiological assessment of aseptic operations have not been defined or associated with historical counts taken in the facility."

Answer key:

1. Endotoxin

2. CFU

3. Cilia

4. Dry ice

5. Halophile

6. FTM, SCD (TSB)

7. Chocolate agar

8. Catalase

9. Taxonomy

10. Pasteurization

ARTICLE REVIEW

An Industry Commentary on Regulatory Issues-Microbiology
Jeanne E. Moldenhauer

PDA Journal of Pharmaceutical Science and Technology
March/April 2001, Volume 56, Number 2

The author has summarized the industry commentary on the regulatory issues into three categories, laboratory methods, inspection issues, handling out of specification test results. Under the laboratory methods the author addresses that though many methods have been revised in the monographs, there still remain methods that need to be revised to use new rapid technologies that could help in obtaining faster and more accurate results and could be more cost effective.
USP intends informational chapters to be guidelines only. However, frequently FDA inspectors interpret these to be compendial methods and expect the user to show equivalency studies for methods other than those in the informational chapters.
"Guidance for Industry on Industry on Investigation of Out-of-Specification Results" is vague and does not provide guidance for assessing microbiological assays
The author emphasizes the need for a guidance for handling OOS results for microbiological test methods.

Bacterial Identification Tips

Tests for differentiating *Micrococcus* from *Staphylococcus*

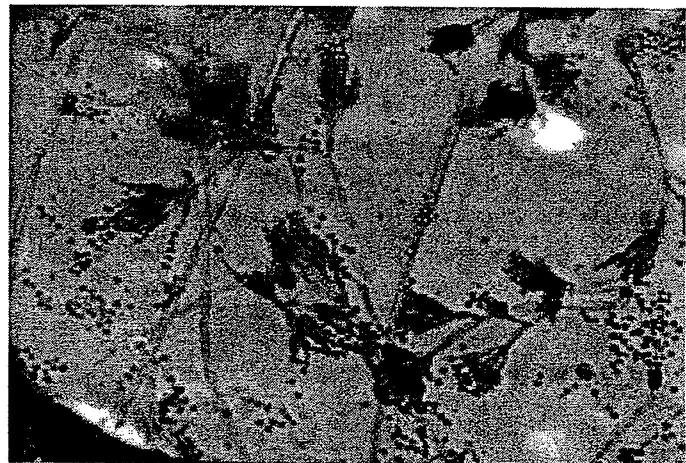
Inhibition of Furazolidone
Staphylococcus is inhibited, *Micrococcus* is not

Resistance to Bacitracin
Staphylococcus is resistant and shows no zone of inhibition, *Micrococcus* is not resistant and shows zone of inhibition

Modified Oxidase Test
Micrococcus is positive to modified oxidase (color change to blue-purple) *Staphylococcus* is negative (no color change)

Lysostaphin
Staphylococcus is positive for lysostaphin (clearing of suspension). *Micrococcus* is negative for lysostaphin

Fungal Identification



Penicillium
Hyphae: Hyaline
Conidiophores branched and hyaline, bearing ampulliform phialides in brush like clusters (Penicilli)
Conidia: round, unicellular slightly rough walled.

Pharmaceutical Microbiology Forum
Membership Application
or
Change of Information Form

MISSION: *The PMF provides a forum for pharmaceutical microbiologists to exchange information on microbiological issues in the pharmaceutical and related industries and interact with the USP and regulatory agencies.*

THIS APPLICATION IS:

A New Member Application	☐
To Update my information, as indicated	☐
Membership Renewal	☐

Mail Application and Fees To:

Pharmaceutical Microbiology Forum
C/o Laura Valdes-Mora
3166 Wood Valley Road
Panama City, FL 32405

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Department:		
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Address:		
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