PMF Newsletter

A quarterly publication of the Pharmaceutical Microbiology Forum

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Letter from the President

Have you noticed a change in the focus of audits of Microbiology Laboratories? In the past, decisions made based on one's knowledge of Microbiology were considered acceptable. These days, the regulatory climate has become stricter (perhaps more knowledgeable of Microbiology) and in turn, the same rules applied to a Chemistry Laboratory are applied to Microbiology. An example would be an SOP that calls for incubating samples at 30-35°. If your incubator registered 29.4°C, are you in compliance? No. Can an organism capable of growing at 30° be able to achieve optimum growth at 29.4°? Yes. However, during an audit, you will be found out of compliance. How can you solve the dilemma of using scientific judgement in conjunction with regulatory compliance? Make your SOP's detailed enough to cover situations such as the one described here. This topic was one of the many topics we discussed during the past PMF General Assembly Meeting and the AAI Microbiology Seminar in Wilmington, NC. There is a summary of the seminar in this issue.

I would like to thank the members of the Organizational Board and the Newsletter Committee for their dedication and commitment to the PMF and its Newsletter. In order to keep this organization useful to you as a Microbiologist, we need your help. How can you help? Send answers to questions that are published, send questions to be answered and answer our questionnaires. There is no need to type your responses or correspondences with us, we accept handwritten documents and letters. Our goal is to make it easy for all pharmaceutical Microbiologists to communicate and exchange information. This is why your involvement is so crucial. Please drop me a note and let me know how we can make this organization more useful to you.

Summary of the AAI Microbiology Series

From April 26-April 28, 1995, the AAI Microbiology series Validation in the Pharmaceutical Microbiology Laboratory, What's New in 1995 was held at the Wilmington Hilton. Below is a brief summary of a few of the sessions. Additional summaries will be included in the Fall newsletter.

Validation of Automated Speciation Systems

An overview of how one approaches validation for this type of equipment was given. The validation team is to consist of the users, QA, PM engineer, Metrology and computer validation specialist(s). You need to perform an IQ, OQ, and PQ of the system. Use of QC organisms recommended by Vitek should be included, supplemented with other ATCC cultures customized to your facility. If you have a baseline of the microbial flora of your pharmaceutical environment, you should test the system with more species of the predominant genera. Accuracy and reproducibility are included in the testing. During accuracy testing, you may run three different culture preparations of each chosen organism. For reproducibility, you may run the same culture preparation in triplicate. *(cont on pg 5)*

Inside This Issue

Container/Closure Integrity Testing

Container/closure integrity testing is required by FDA for all parenteral products.

The USP sterility test has been used to demonstrate container/closure integrity on long stability samples. But, passing a sterility test does not prove that the container/closure integrity is acceptable. If the product is bactericidal or contains preservatives,it may kill any microorganisms that may enter through a defective seal.

Several factors affect container/closure integrity: glass dimensions, glass defects, closure formulation, closure defects, capping machine, product terminal sterilization, material identity and the container internal pressure. This summary only covers aspects related to microbiology.

Container/closure integrity is tested by physical and/or microbiological methods. Choosing the appropriate test depends on the product's storage and shipping conditions.

Microbiological Test Methods

Most microbiological methods are performed with media filled containers. The media normally used to fill the containers is Tryptic Soy Broth.

The tests are performed under the following conditions:

a. Static - aerosol challenge
b. Static - immersion challenge
c. Static - ambient challenge
d. Dynamic - immersion challenge

Static aerosol challenge is performed by exposing sealed media filled containers to periodic challenges with a challenge organism. Vials are usually incubated inverted for the duration of the test. This is probably the most difficult method to follow, due to the difficulties of preparing the aerosol challenge. The challenge may vary from minutes to hours.

Static immersion challenge is probably the most common method performed in the pharmaceutical industry. Using this method, media filled vials are immersed, usually inverted, in media spiked with the organism. This type of test is a more severe test than the aerosol challenge.

Static ambient challenge: Some companies incubate media filled vials at ambient conditions and monitor them periodically for evidence of microbial growth. Usually the incubation time is the same or longer than the product shelf life. This method could be combined with the immersion test, where vials are immersed in spiked media at specific intervals, and then checked for growth. Vials are usually incubated for the duration of the test.

Dynamic immersion challenge is performed by subjecting media filled vials to periodic challenges by immersing them in spiked media, while simultaneously adding the stress of pressure and/or vacuum and sometimes different temperatures, if warranted by the normal conditions of product storage. Vials are usually incubated inverted for the duration of the test. This method is more severe than the static immersion test performed at ambient conditions.

Challenge Organisms

The following organisms are usually used for container/closure integrity testing. Usually only one organism is used for the challenge. The concentration of the challenge organism varies from 10^6 to 10^8 cfu/mL. The test incubation temperature may include 2-8°, 20-25°, and 30-35° C or a combination of temperatures.

Pseudomonas aeruginosa
Pseudomonas diminuta
Escherichia coli
Serratia marcescens
Bacillus subtilis

Summary

Proving a product's container/closure integrity involves more than conducting sterility testing while the product is on long term stability. The conditions of the product's manufacture, packaging, storage and shipping must be evaluated to determine the appropriate test method, challenge organism and incubation period to use to assure container/closure integrity.

Protocol for Closure/Integrity Testing

This protocol will outline a method for evaluating the microbial barrier properties of 2x heated water sterilized 10ml amber glass vials. This protocol will also evaluate the microbial barrier properties of the cap when secured on the vial at the minimum and maximum pressures, following exposure to 2 consecutive heated water sterilization cycles. Samples capped at minimum and maximum pressures will be exposed to 2 consecutive superheated cascading water sterilization cycles prior to the closure/integrity test. Samples will be evaluated initially following 2x sterilization (time zero) and after storage at ambient conditions for nine and twelve months. Additional samples will be evaluated following exposure to accelerated aging conditions for two and three months. Sterilization will be conducted at maximum parameters.

The sample size will be determined by using Military Standard 109E, Level II. Clear glass vials of equivalent dimensions will be used instead of the amber glass vials routinely used in order to facilitate viewing of the microbial growth during the sterility test. Equivalency of the vials has been established by the vial manufacturer.

A minimum of 2150 10mL clear glass vials containing 10mL of Tryptic Soy Broth will be sealed and exposed to 2 heated water sterilization cycles. These samples will consist of the following parameters:

1. A minimum of 215 samples capped at minimum downward pressure
2. A minimum of 215 samples capped at maximum downward pressure
3. A minimum of 430 samples capped at minimum downward pressure and exposed to accelerated aging. 215 of each will be stored on their side for 2 months and three months at 40°, ±1°C.
4. A minimum of 430 samples capped at minimum downward pressure and sterilized at a 2x sterilization cycle. Samples will be stored for accelerated aging for 2 and 3 months, as in #3.
5. A minimum of 430 samples capped at minimum downward pressure and exposed to accelerated aging. 215 of each will be stored on their side for 9 and 12 months at ambient storage (25° ±2°C).
6. A minimum of 430 samples capped at minimum downward pressure, exposed to 2x sterilization cycle. Samples will be stored for accelerated aging as in #5.

Procedure for microbial testing of samples will be performed as follows:

Growth promotion/Bacteriostasis will be performed to demonstrate that there are no bacteriostatic characteristics associated with the package and sterility test medium, and that the medium used in the sterility test will support the growth of low numbers of the indicator organism. Growth promotion testing will be performed on samples capped at each seal parameter and aging sequence. Samples will be pre-incubated for 48 hours at 30°, ±2°C prior to the initiation of the study.

Testing Sequence

Prepare a suspension of *Serratia marcescens* at a concentration of less than 100 organisms at a defined volume. Inoculate the medium in 4 separate vials each capped at minimum and maximum pressures with less than 100 organisms. Incubate the vials with a negative control at 30°, ±2°C for 7 days or until growth is observed. Verify the inoculum by standard plate count. If no growth is observed in the inoculated vials, repeat the study with new vials. Evaluate the medium for growth promotion capabilities with less than 100 organisms *Bacillus subtilis* ATCC6633 and *Candida albicans* ATCC10231 in separate duplicate vials. Verify the inoculum concentration of each organism by standard plate count method. Incubate the samples at 30°±2°C with a negative control for seven days, or until growth is observed. If no growth is observed, the media is unacceptable for use and the challenge cannot be conducted on the submitted test vials. If growth is observed with the above organisms, repeat the study with a new *Serratia marcescens* suspension and new test vials. If no growth is observed with the retest, select an alternative organism such as *Pseudomonas diminuta.*

Repeat the growth promotion study for each set of sample conditions. *(cont pg 4)*

(Closure Integrity cont.)

Microbial Ingress Testing will be completed following the acceptance of the growth promotion study. Inoculate the indicator organism into at least 100mL of Tryptic Soy Broth. Incubate for 24 hours at 30°, ±2°C. Determine the initial concentration by standard plate count. Fill the vessel with a known volume of deionized water in which the test racks can be fully submerged. Add 1.25 mL of Butterfield's stock solution per liter of deionized water. Agitate the vessel to distribute the buffer. Transfer an adequate volume of the indicator organism suspension previously prepared to provide a theoretical concentration of at least 10^5 cfu/mL. Agitate the vessel to distribute the suspension. Place 100 samples into the test racks with the vials upright and 100 vials into the test racks in the inverted position. Place the test racks into the vessel. Secure the samples to insure that they remain fully immersed in the microbial suspension. At ten minute intervals, manipulate the vessel to maintain the microbial suspension. After fifteen minutes, aseptically transfer a 5 mL aliquot of the suspension to a sterile test tube. Determine the concentration of the suspension by standard plate count. After thirty minutes, remove the samples from the vessel and dry under laminar flow. Aseptically transfer an additional 5 mL aliquot to a sterile test tube and determine the concentration of the suspension by standard plate count. Identify and inoculate 5 samples with 0.1 mL of the suspension to act as positive controls. Identify 5 samples unexposed to the microbial challenge as negative controls.

Transfer the vials to the incubator and incubate the vials inverted at 30°, ±2° C for seven days. Incubate three positive control samples in the inverted position and two positive controls samples in the upright position with the test samples. Incubate two negative controls in the inverted position and three negative controls in the upright position with the test samples. Examine all vials for growth of the indicator organism at the end of the incubation period. Repeat the microbial challenge in the above manner for samples prepared at each set of conditions.

In order for the test to be considered acceptable, the following conditions must be met:

1. All growth promotion test samples must demonstrate growth of the indicator organism
2. All positive control test samples must be positive at the end of the incubation period
3. All negative control test samples must be sterile at the end of the incubation period
4. All test samples must be sterile at the end of the incubation period
5. Each microbial challenge must be at least 10^5 cfu/mL

Calendar of Events

The Calendar of Events is provided as a service to PMF Newsletter readers. Submission of complete and accurate information will be published on a space-available basis.

Sept. 7-8/Oct. 18-19, 1995, *PDA Limulus Amebocyte Lysate (LAL) Test Technology and LAL Workshop,* Malvern, Pennsylvania and San Juan, Puerto Rico

Sept. 11-14, 1995, *ISPE Philadelphia Seminars,* Desmond Great Valley, Malvern, Pennsylvania

Sept. 14,1995, *PDA-Sterility Testing in a Barrier Isolator,* San Diego, California

Sept. 18-20,1995, *PDA/FDA Joint Conference,* Bethesda, Maryland

Sept. 18-20, 1995, *Pharm Tech Conference and Exhibition,* East Brunswick, New Jersey

Oct. 10-13, 1995, *ISPE Raleigh Seminars,* Raleigh Marriott Crabtree Valley, Raleigh, North Carolina

Nov. 2-4, 1995 *1995 PDA Annual Meeting and Exhibition,* Philadelphia, Pennsylvania

Future Topics

The purpose of the Newsletter is a sharing of information among Microbiologists. Your contributions to *PMF Newsletter* are needed in the form of short articles, letters to the Editor, comments, or suggestions. Please direct your correspondence to *PMF Newsletter,* c/o L. Valdes-Mora, 1206 North 23rd Street, Wilmington, NC 28405 [Tel (910) 251-6786 or FAX (910) 251-6755. Submit any articles with your name and phone number in case we need to contact you. Your name and company will not appear without prior written authorization.

(Micro Summary cont)
The software validation is a very time consuming part. It takes approximately 160 hours to complete. You may use 25 to 100 organisms for the validation. Every organism will be run six times during precision and accuracy. Therefore, the number of organisms needs to be limited. The computer specialists will work with the system to determine if the calculation of probabilities for each bionumber is accurate. A total of 319 bionumbers were tested. Of these 302 produced the expected results and 17 bionumbers produced aberrant results. Protocols were available at the seminar for attendees to review.

Validation of Isolation Technology Systems

Dr. Agalloco clarified that barrier and isolation are different terms. Isolators are absolute in terms of separating 2 areas, 2 environments, while barriers are not absolute. The big issue with isolators is how to get the materials into them. Main validation concerns are how to clean powder residues and also how to protect media from a sterilant such as vaporized hydrogen peroxide(VHP) in order to prevent false negatives. Open isolators are used for manufacturing, while closed isolators are suitable for sterility testing. The closed isolator is operated as a sealed system at all times, has no exchange of air with its surroundings, may be sterilized infrequently and environmental monitoring does not need to be as frequent as in a conventional clean room. Agalloco predicts that conventional clean rooms will be obsolete 5-10 years from now. Isolators are 25% the cost of a conventional clean room and their operation is 10-15% that of rooms. If you will be performing sterility testing you need to perform a container/closure integrity test to check samples, media and equipment, perform bacteriostasis/fungistasis in the isolator, establish environmental monitoring and determine the frequency for sterilization. Most people do it once a month. If you are using the Steritest system, this must be sterilized with VHP not more than 2 times. The Steritest as it is constructed to date will retain HP and can give you false negatives.

Dry Heat Ovens

The depyrogenation oven must be subjected to IQ, OQ and PQ. After IQ is completed, you must officially document any changes made to the equipment as per your company's change control SOP. During OQ, you should check the functionality of alarms (electronic or mechanical), door interlocks, belt speeds(for production), cooling coils, blower speed, and heater draw. You should perform chamber mapping studies. During the PQ is when the Microbiologists get involved. Prior to PQ, during protocol design, you need to determine which areas are to be spiked (hardest ones to depyrogenate) and how many log reductions are required to consider the test acceptable. Whenever you inoculate material, you need to determine endotoxin recovery. The recovery must be consistent. If you get 90% recovery, this is good. However, if you always get 20% recovery, this is acceptable, because it is consistent. Prior to executing your validation, based on the acceptance criteria, the LAL methodology needs to be chosen. If your criteria is a 3 log reduction, but your methodology sensitivity can only calculate a 2.5 reduction, you will waste your time during validation. You should spike between 10 to 20 containers. Spiked items are good for up to a year, if you check to determine that the level of endotoxin is still at an acceptable range. During depyrogenation validation, a minimum-maximum load approach is not used. Usually only maximum loads are validated.

For the assays, you need to qualify your analyst and your reagent. During this qualification, the results cannot be averaged. During the testing section, averaging results is acceptable.

Clean Steam

A former MCA (Medicines Control Agency) inspector, while performing a mock inspection for a PMF member firm, noted that the European regulators require manufacturers to test clean steam systems for three parameters. These required tests are: 1) noncondensible gases, 2) superheat, and 3) dryness. PMF has a copy of the auditor's suggested procedures including specifications for interested PMF members. The literature source is unknown. For a copy, please contact Laura Valdes-Mora.

QUESTIONS/ANSWERS

1. Temperature Monitoring
   - What tolerances should we use?
   - At 32-35°C: Which is the main system, the temperature recorder or the thermometer, if both systems operate simultaneously?
   - Is 37.8° C an Out of Specification (OOS) result?

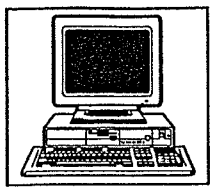
Per an ex-FDA Employee
   - The FDA expects you to have a second point of reference in your incubator.
   - A glass thermometer and 7 day chart must be recorded daily. Why? You need to know before 7 days that you are out of compliance (±0.5° or ±1.0°C)
2. Does anyone have experience with Japanese Pharmacopeia (JP)?
3. Environmental Monitoring
   - If rooms are kept at a low temperature, why incubate plates at 30-35°C? Most people indicate they use a dual temperature, 30-35°C for two or three days, then change to 20-25°C for five days.
   - If the same disinfectant is used for a long time, the flora may change over a period of time and a different disinfectant may be needed.

WANTED

Due to the recent death of a board member, the PMF is looking for an outgoing individual who would like to be a part of the Organizational Board. The Board Members communicate various times per quarter to set and approve the newsletter. The Board also meets once a year to evaluate past performance and set new goals for the organization. We are looking for a pharmaceutical or biotechnology microbiologist who will volunteer for this.

If you are interested, please call Laura Valdes-Mora at (910) 251-6786 or FAX her at (910) 251-6755. She will contact you and give you additional information. Nominees will be taken to the Board and the new member will then be chosen. The deadline is September 15, 1995.

ON LINE SERVICES



Three new services are claiming to answer questions for the pharmaceutical microbiologist. Here is a list of the services. For information, contact each service directly or read this newsletter for further information. PMF is not related to any of these services.

PharmComm, Inc.
Bruce C. Rudy, Ph.D.
1410 Commonwealth Drive, Suite 225
Wilmington, NC 28403
Voice: (910) 256-4146
Telefax: (910) 256-8245
Internet: rudyb@pharmcomm.com

MicroBio Online
An electronic bulletin board dedicated to providing networking opportunities, forum and information exchange in the field of microbiological quality control. Call (303) 384-3535(BBS) for a free online tour or (303) 279-4643 (VOICE) for more information.
Information as seen in *Microbiological Update Newsletter.*

The Microbiology BBS
Scott VW Sutton, Ph.D.
P. O. Box 173065
Arlington, TX 76003
(817) 557-0330 (data)
Email microbiol.org.unicomp.net
SYSOP: Sutton@microbiol.org.unicomp.net

Below is information on *The Microbiology BBS.* Information on other systems will be in upcoming newsletters.

An electronic bulletin board service (BBS) specifically aimed at the concerns of the professional biologist in industry, academics, and private practice is now available. *The Microbiology BBS* is available via modem (N/8/1) at (817) 557-0330. *(cont pg 6)*

(BBS cont)

The objective of this service is to act as a repository for computer files of interest to microbiologists, healthcare practitioners, and interested laypersons. Examples of these files range from programs designed by individual investigators to demonstration programs supplied by vendors. A second major function of this service is to facilitate communication among workers. This BBS allows the convenient exchange of mail, files and information among users. In addition, different forums are presented providing a structure for the discussion of specific topics. Finally, the BBS will serve as a source of information for the interested worker with the development of on-line databases covering upcoming meetings and training opportunities, contract testing facilities, and job opportunities.

The Microbiology BBS is run using a new software package - Worldgroup - by Galacticomm. This software is unique in that it allows access to the BBS not only by standard computer communications software, but also provides a complete Windows-based communications program. The program can be downloaded as "MICROBBS.EXE." Paid subscribers may have the program mailed to them by leaving a message to the sysop. Of course, the BBS can be enjoyed by using a standard communications package.

This service has received international recognition. *The Microbiology BBS* has been described in the newsletters and journals of the Parenteral Drug Association, the American Society for Microbiology, the Society for Industrial Microbiology, and the Canadian Society of Microbiologists.

The Microbiology BBS offers a unique combination of services. Full service internet E-mail capabilities are provided free of charge to all subscribers. The bulletin board service also offers "state of the art" scientific and medical knowledge to both the practitioner and the concerned layman. This potential comes from the unparalleled combination of Information networks available. In addition to Internet, *The Microbiology BBS* provides access to specialized private networks including FidoNet, The HealthCare Network and AegisNet.

Access to *The Microbiology BBS* is available on several levels for the individual user. The first level is a demonstration access. This level allows any caller access to Aegis.Net, registration, the "Trial Account" questionnaire and viewing of files. The next level is a trial account. This account is granted after filling out the "Trial Account" questionnaire, and allows full access for three weeks. The final level is granted to subscribers who have paid for a subscription to *The Microbiology BBS.*

Corporate opportunities are also available on *The Microbiology BBS.* Businesses may set up Email accounts in an inexpensive fashion to provide communication with other businesses or individuals. Private concerns may host and control access to a forum of interest to their business interest. Job postings may be accomplished on the InterNet, HealthCare Net, and FidoNet through the BBS. Finally, an InterNet location can be established through *The Microbiology BBS* and maintained by our staff. Client organizations realize the benefits of an InterNet presence, and a BBS presence, without the capital expense and personnel concerns associated with going on-line from within the organization.

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

The information contained in this newsletter are the professional opinions of our members and do not represent the policies or operations of any corporation or government agency to which members may be associated. *PMF Newsletter* is intended to serve as an open forum and confidentially will be maintained. The information in *PMF Newsletter* is solely for information purposes and is developed from sources believed to be reliable. Statements expressed constitute current opinions derived through analysis of available information and professional networking. The *PMF Newsletter* cannot make any representations as to the accuracy or completeness of the information presented and the publisher cannot be held liable for errors.

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Address Correction Requested