PMF Newsletter

A publication of the Pharmaceutical Microbiology Forum

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1994 Annual Meeting

The PMF Annual Meeting will take place at the Wilmington (NC) Hilton on Sunday, April 24, 1994 from 6:00 PM to 7:00 PM followed by an opening seminar cocktail. The Organizational Board will meet earlier in the day at 11:00 AM for a working lunch. The Organizational Board will also meet Sunday with the Newsletter Committee at 2:00 PM and the USP/FDA Liaison Board at 4:00 PM.

The General Assembly meeting will include an introduction to interested people, a summary of accomplishments, election of officers (president, vice president, secretary, treasurer), a call for volunteers for the subcommittees, and an open floor discussion on topics the attendees feel should be addressed by the PMF. Topics for discussion may be submitted in advance in writing and anonymously. The PMF By-Laws will be available at the Annual Meeting. The Focus Groups will be completed and their goals will be traced at the Annual Meeting. For more information on the Pharmaceutical Microbiology Forum, please contact L. Valdes-Mora, AAI, Inc., 1206 North 23rd Street, Wilmington, NC 28405 [Tel (910) 251-6786 or FAX (910) 251-6755]. ■

Inside This Issue

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AUTOCLAVES: The Ins and Outs of Purchasing a New One

Contributed by a PMF Member

As Microbiologists most of us have used but may not have been involved in the purchase or installation of autoclaves. This can be a very frustrating but valuable experience. Autoclaves are a major long term investment. They can be rebuilt by stripping off all the piping, pressure testing the chambers and adding new piping and control systems. Therefore the money spent on a good quality unit is money saved.

My company agreed to replace the autoclave that resided in the Microbiology Laboratory. Because we are a small growing company it was decided that this autoclave should also be a back-up for the manufacturing sterilizer, which is responsible for toxicology and early clinical sample production.

Therefore we needed an autoclave that fulfilled cGMP requirements and tried to decide what these were. After talking to validation people, sterilizer vendors and other microbiologists we decided that our autoclave should have the following:

1. a electronic control system that will maintain ±0.5°C temperature in the chamber
2. a clean steam source
3. a stainless steel chamber
4. stainless steel piping for the clean steam

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Autoclaves *(continued from page 1)*

5. a validation port in the chamber
6. a media probe
7. a pre-vacuum cycle for which the pulses can be programmed
8. a system that allows cycles and alarms to be recorded on paper
9. an impact printer

We needed a 20" x 20" x 38" size, a relatively small one, in the world of sterilizers. Our pilot plant autoclave runs a wrapped cycle that needs pre-vac to insure that all the air is removed before the sterilization cycle starts.

As a clean steam source, it was recommended that we use a steam to clean steam generator, also known as a hydrogenerator. This is an accessory that is added to the autoclave. It uses house steam in the jacket of the generator to heat the DI water within the chamber of the generator. This produces clean steam in the chamber of the generator which is then piped through stainless steel pipes to the chamber of the autoclave. Stainless steel is necessary because the DI water will remove ions from other metals and cause excessive corrosion of the pipes. These ions may also cause heavy metal contamination, depending on the composition of the pipes. The stainless steel should be 316 grade throughout the vessel. There are other grades of stainless steel and these should not be used interchangeably.

The steam to clean steam generator is less expensive to purchase and to operate than a standard clean steam generator because it uses an in-house steam source available from the boilers within the facility.

The validation port allows thermocouples to be inserted through a Conax connector into the chamber. This is useful during validation studies and saves damaging the thermocouples if they are not run through the door. If a chamber is built without this port, it is not usually added later without addressing some major safety concerns.

The impact printer is necessary, especially if the tapes are to be stored for any period of time as a GMP requirement, thermopaper can turn dark and the print becomes illegible.

If you plan to make media in the autoclave, a media probe is recommended. The autoclave can be programmed to control the cycle either from the probe temperature or accumulated F_o. The probe is calibrated during the validation of the sterilizer and is used during the validation while running the liquid cycles.

From experience, I highly recommend that the installation be performed by the manufacturer or its representative. Do not save a few dollars by having it done by someone else. It can end up costing many thousand of dollars. In one instance I know, there were issues that would have cost the purchaser $30,000 more had the machine not been installed by the manufacturer. In another instance there was $8,000 of additional expenses that had to be absorbed by the purchaser, because they had some one else do the installation and it was not done correctly. This was not detected until the unit was validated and one of the alarms was not working.

You may require some guidance from the sales representative as to what features are available on their sterilizers. It is best to work with someone experienced with GMP autoclaves and I would insist on it. A sales rep who sells primarily to hospitals may not know some of the current requirements, real or potential, for the pharmaceutical industry.

Once the autoclave is purchased and arrives, the installation is scheduled. It is wise to go over everything after it is installed. For example, installers for the manufacturer of our unit came from two other states and did not know the state codes for drains and they had to be replumbed. Also they used PVC pipe to connect to the polypropylene from the DI water system. The manufacturer made the corrections at its expense.

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Irradiated RODAC® Plates: A Response

Contributed by a PMF Member

Other than the sterile plate and long shelf life mentioned in the article, the irradiated plates also have the advantage of proper fill volume which is something that not every in-house lab can achieve. We have had some problems though with our purchased plates: 1) in a recent lot we found some white matter that was subsurface and looked like colonies. However, the manufacturer determined that it was precipitated lecithin. 2) we also found a small fly in the bottom of one plate (dead and sterile I suppose, but of concern nevertheless).

I also want to address two concerns mentioned in the article. The first dealt with the issue of whether irradiation would affect the growth promoting properties of the medium. While I agree from a purely scientific view that this is a possibility, I think one should keep in mind that the use of RODAC® plates is not intended to pick up every organism present in the environment but rather to pick up trends towards higher than usual flora in a location. Therefore, I believe that if you stick with the same manufacturer's plates every time, you can make a case for changes in microbial load between samplings being important rather than questioning if every bug present in the area was capable of growing on the medium. Additionally, for user quality control (I hate to use the word validation for something such as a plate), we rely on the manufacturer's Certificate of Analysis and also test each lot of plates as follows: We use the NCCLS document M22-A [National Committee for Clinical Lab Standards, 771 East Lancaster Avenue, Villanova, PA 19085 (215) 525-2435] which is a compendial test procedure for clinical lab media (so I think the FDA would be hard-pressed to cite us but you never know) and then test the medium (TSA) with a Gram-positive coccus, Gram-negative rod, and a yeast (all of known ATCC varieties). This way I believe most organism Gram-stain types present in an environment could be shown to be capable of growth.

The second issue I want to address relates to the outer packaging of the plates. Although I would agree that the outer packaging being cardboard or whatever, may be contaminated with molds or anything else, I would point out that no matter what the packaging is, there is ALWAYS going to be an outside which is exposed to the environment. The idea of the triple-wrapped plates is that you remove the outer bag within the least clean of your areas and then you have a sterile wrapper which you will be exposing to the next cleaner area. I just wouldn't bring the box to the clean area with you. I don't know any other way around it. If you made your own plates, you would still be faced with the same issue and then some.

As far as drying of the plates, we usually take them out overnight and leave them on the bench. Since the manufacturer as well as we have done growth studies to show that the plates will support the QC test organisms at room temperature for up to 6 weeks, I am not concerned about leaving them out on the bench overnight. If we are in a hurry, we have also taken the plates and placed them inverted into a 35-37°C incubator 1-2 hours before needed and have not had a problem with coalescing colonies. This was not the case when we used to make our own plates.

Author's note: The media supplier has since gone out of business and was taken over by another company, who was already selling triple-bagged irradiated plates. ■

Future Topics

Contributions to *PMF Newsletter* are welcome and may be 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. ■

Overview:
Biological Indicators

Contributed by a PMF Member

In some pharmaceutical manufacturing companies there is a concern that steam-in-place (SIP) and process sterility validations must pass at any cost. One such company would add time on to the cycles to compensate for biological indicator (BI) spore strips that had high D-values. They did not use the same range of D-values, but would take whatever the vendor sent and change its cycles to accommodate the different lots of BI's. I would like to present my interpretation of the following from: *Pharmacopeial Forum* in-process revision Sept-Oct. 1992.

"<1035> A biological indicator is a characterized preparation of specific microorganisms resistant to a particular sterilization process. It is used to assist in the qualification of the physical operation of sterilization apparatus in the development and establishment of a validated sterilization process for a particular article, and the sterilization of equipment, materials, and packaging components for aseptic processing. It may also be used to monitor a sterilization cycle, once established, and periodically in the program to revalidate previously established and documented sterilization cycles.

A biological indicator is in one of two main forms, each of which incorporates a viable culture of a known species of microorganism. In one, the spores are added to a carrier (disk or strip of filter paper, glass, or plastic) and packaged so as to maintain the integrity of the inoculated carrier but, when used appropriately in the individual immediate package, it allow the sterilizing agent to exert its effect. In the other, the spores added to representative units of the lot to be sterilized (inoculated product) or to similar units (inoculated similar product).

If the material to be sterilized is a liquid, and if it is not practical to add a biological indicator to selected units of the lot, viable spores may be added to a simulated product but in such a way that the resistance of the simulated product to the sterilization process does not differ from the resistance to sterilization of the product to be sterilized. In either case the product or simulated product should not adversely affect the resistance characteristics of the added viable spores.

The effective use of biological indicators for the monitoring of a sterilization process requires a thorough knowledge of the product being sterilized and its component parts and, for an overkill approach, at least a general idea of the probable types and numbers of microorganisms constituting the microbial burden in the product immediately prior to sterilization."

I am interpreting this to read that a cycle is developed and the BIs are used as indicators. BIs approach a maximum practical all-survivor limit of 12 minutes at 121° C. This corresponds to an F_o of 12. BIs that can survive sterilization at 121° C for up to 12 minutes exhibit high D values and approach a limit relative to the number of organisms that can practically be inoculated or found naturally on a carrier material. Thus, the maximum process lethality (F_o) that can be practically measured using BIs is 12. This fact concurs with the PDA (1980) recommendation of an F_o of 12, the minimum article lethality, for steam sterilization processes where bioburden and organism resistance studies are not performed on heat stable materials to be sterilized.

Validation of steam-in-place cycles or product sterilization cycles are performed after the sterilization process has been developed. There should be an understanding of the bioburden levels and the resistance of the organisms isolated from the product and from the environment. A low bioburden containing heat sensitive organisms requires lower D-values and a shorter cycle time to achieve a satisfactory sterility assurance level. If the product and/or environment contains high levels of contamination and/or heat resistant organisms; a higher D-value BI should be chosen to assure that a satisfactory kill has been obtained.

It is advisable to choose a D-value range, i.e. 1.5-1.9 or 2.0-2.5, for validation work and stay within that range in future revalidation. If there is a failure on revalidation, it may be due to some unknown cause that would come to light in the failure investigation.

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Autoclaves *(continued from page 2)*

The sterilizer had been shipped without sides to the chamber and the manufacturer had to build a stainless steel cabinet for the equipment. They had to install an expensive heat exchanger to correct another problem.

The validation team discovered the manufacturer had mixed 304 and 316 gauge stainless steel, the chamber was not level, there was brass piping in areas that should have had stainless steel, there was a brass drain in the chamber and we had a problem passing the vacuum leak test.

It was 10 months later that the manufacturer finally determined that there was a design problem between the steam to clean steam generator and 2 months after that the software was not appropriate for our machine. This sterilizer and the steam to clean steam generator were a new design and apparently hadn't had all the bugs worked out. It cost 16 months of total frustration for us and the manufacturer. We were not able to use the sterilizer while they chased leaks, installed an expensive steam separator, repiped and changed software.

Validation is a process that determines whether the equipment meets the manufacturers specifications. For an autoclave this includes checking that all the alarms are working properly, that the chamber can maintain the temperature profiles and that the system can maintain vacuum or pressure without leaking. Without proper validation we would not have known that our sterilizer was not performing as we expected. ■

Coming in PMF

Current Issues in Microbiology, Methods Development, Regulatory Trends, Book Reviews, Career Development, Job Opportunities

Formation of a GC Microbial ID User Group

There are now greater than 50 pharmaceutical companies who utilize the GC microbial ID system for microbial identifications. Pharmaceutical companies interested in forming a GC user group should contact Dr. Myron Sasser at MIDI (302) 737-4297 by April 29, 1994. ■

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.

1994

April 25, *1994 PMF Annual Meeting*, Wilmington, NC (910) 251-6786

April 25-26, *Third Annual Applied Analytical Industries, Inc. (AAI) Microbiology Seminar Series*, Wilmington, NC (910) 392-4694

May 22-26, *American Society for Microbiology General Meeting (94th)*, Las Vegas, NV (202) 737-3600

June 13-15, *BioPharm Conference*, San Francisco, CA (503) 343-1200

July 9-13, *American Society for Virology Scientific Meeting (13th)*, Madison, WI (608) 262-9880

October 4-7, *Interscience Conference on Antimicrobial Agents & Chemotherapy (34th)*, Orlando, FL (202) 737-3600

The Pharmaceutical Microbiology Forum would like to express its appreciation and thanks to Applied Analytical Industries, Inc. for their support.

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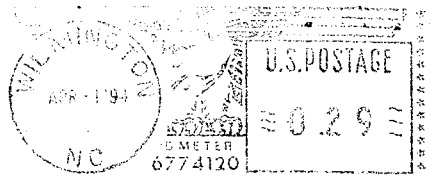

Address Correction requested

BIs *(continued from page 4)*

If spore preparations are purchased commercially, the vendor will usually have ranges available within a given population spores. For example, you can request a 10^6 population with a D-value range of 1.5-1.9.

If the material to be sterilized is a liquid, it is not appropriate to add spore strips directly to the liquid. The preferred method for liquids is to add a suspension of spores, either directly or in a self-contained system, i.e. ampule, to the product or to a representative of the product. For example, when autoclaving media a BI ampule is suspended in the middle of a bottle of media or a representative bottle of water during the sterilization cycle. When a sterilization validation is performed for a product made in a batching or mixing vessel, a thermocouple or other means of monitoring temperature and accumulated F_o are attached with the BI ampules in the liquid portions and the BI strips in the interior areas not immersed in liquid.

Research indicates that spores strips in glassine envelopes need less heat than those without the glassine envelopes. Commercial products generally test their BIs as a system. Different materials may change the heat penetration profiles. Therefore, if the method of attachment of the BIs increases the resistance of the spores to heat penetration, that should be validated.

BIs are indicators for the system and the sterilization cycle should not be changed to accommodate the resistance of the indicators. The sterilization cycle time should be increased if more resistant environmental organisms or higher bioburden levels develop in the product or environment. ■

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