PMF Newsletter

A quarterly publication of the Pharmaceutical Microbiology Forum

Winter, 1994

Answers to the Questions From the Summer Issue

Question 1. Have you had an autoclave problem? If so how did you solve it?

Answer: We experienced two problems with our Tuttnauer/Brinkman Steam Sterilizer, Model 3870EP (with electronic printer)

Problem #1) When pushing water inlet key there was no flow of water into the autoclave chamber. The buzzer was on, and there was a failure to start the autoclave for sterilization. Upon inspection it was found that the culture media overflowed from the media container during the sterilization process. The media (agar) had clogged the sterilizer strainer along with the solenoid valves. This problem was resolved by cleaning the strainer, solenoid valves, and line with pressurized air and water.

Problem #2) When starting the sterilization cycle the temperature did not reach 121°C, and the pressure did not increase to 15 lb. This fails to meet the sterilization requirement. The problem was corrected by releasing the safety valve two or three times. Then start the sterilizer, and wait until it reaches maximum pressure. Using a screw driver pull the safety valve to release the steam. By repeating this two or three times all of the trapped air can be removed, and the clogged jet can be cleaned.

Question 2. How do you test clean steam?

First Answer: We sample selected "critical sites" on a rotating weekly basis. These sites were selected on the basis of whether they are located on the supply line to a production autoclave, post steam generator, or at the end of a long leg of piping (our system is not a circulating loop). Other available ports in the system are not tested. To collect samples we use an HCI Model A-500 Sanitary Heat Exchanger. These can be hard piped which is the preferable method. They can also be mounted on a portable cart with a heavy duty garden hose to carry the coolant. The preferable coolant is glycol, but municipal water can also be used. Several liters of condensate can be collected in under 15 minutes.

At the Spring FDA-PDA Conference on Environmental Monitoring in Bethesda, MD the issue of sampling clean steam was raised. Most people felt that it is not necessary to sample clean steam in a "properly designed" system since typically

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the supply to the generator is purified water. Purified water is already tested to USP specifications for chemistries. One does not expect bioburden to survive in live steam so there is nothing to test. Others recommended that perhaps testing of specific locations be done on a quarterly basis. Most people indicated that they were not testing at all.

Second Answer: The quality attributes of a clean steam system are equivalent to those of a water for injection system. Samples must be available for chemical and microbiological analysis. Clean steam, also known as pure steam, is condensed to the liquid state by employing a condenser. Pure steam condensers are available commercially, or they may be fabricated from 316 L stainless steel tubing and sanitary fittings. One end of the coil of tubing (the inner tube) connects to the steam sample port with a tri-clamp. The other end is directed into the sample container. The exterior tubing inlet port is connected via tubing to a cold water source, and the outlet port is directed toward a drain. Allow the cold water to flow through the unit first to chill it prior to turning the sample valve on.

Extreme caution should always be exercised when working with steam. Appropriate gauges should be checked to determine steam pressure conditions.

The pure steam sample apparatus is sterilized by autoclaving. Biological indicators are employed within the condenser coil to verify the sterility of the sampler.

Pure steam generators should be equipped with a pure steam condensate sampling system utilizing a condenser coil with circulating cooling water. The water cools the steam to a distillate when needed for chemical, and microbiological testing.

Question 3. How do you do sterility tests for mammalian cell cultures?

Answer: There are two approaches to testing mammalian cell cultures. The first approach consists in testing the cell culture media. If medium is found to be sterile it is assumed that the cells are also sterile.

The second approach consists of immersing the cells into the sterility test media as described by the compendia you are going by (USP, BP, or EP). These compendia usually call for a 20 or 40 container test, or a test of 10% of your total cell line vials. Half of the content of each vial is transferred independently into 100 ml of Soybean Casein Digest Broth. The second half of each vial is transferred into Fluid Thioglycollate Broth. During the incubation time of 14 days you will see turbidity. To determine if the turbidity is caused by a microbial entity or by the mammalian cells, subcultures into the broth and a loopful of the most turbid area is transferred to a plate. The subcultures are incubated and checked for various days. The length of the incubation time may vary depending on the possible contaminant, and should be well explained in your SOP. The incubation temperature should be the same as the one used for the original broths from where the subcultures were made.

Question 4. Have you experienced variability in kill times for biological indicators from different vendors with the same D-value?

Answer: No, but I am not surprised. The D-value is guaranteed by it's manufacturer to be reproducible within 20% of it's value (per USP). The variability should be lower if the same BIER (Biological Indicator Evaluator Resistometer) vessel is used.

Greater variability may be observed when other BIER units are used. Also note that during D-value calculations, population determinations are needed. Population determinations contribute to the highest variability from the way the biological indicators are processed to the lot of media used, type of media and vendor of media used. In addition, the inherent variability of the technicians plating skills may produce a population different from the one determined by the manufacturer. In turn the D-value may or may not be affected depending on how far apart the two population determinations are. Due to all of these factors mentioned, the 20% variability range may not seem as wide after all.

One very important aspect of working with biological indicators is to understand what you are getting, what has a D-value at 121°C of 2.0? The value given by a manufacturer is for the biological indicator itself, which in reality is a multicomponent entity or system. The D-value is not of spores only. If you have a strip, the D-value includes the resistance of the filter paper where the spores are embedded, the resistance of the spores themselves, and the resistance to the glassine envelope (blue wrapper). If you purchase a strip and take it out of the envelope, you have changed it's D-value because you removed one of it's components. The new D-value can be higher or lower than what the vendor is certifying. Unless you test the naked strips by themselves in a BIER unit you will not know the correct D-value. This is where two different vendors can really show significant differences, even when the D-value in the package was the same.

The same applies to a self contained biological indicator, if you remove the strip or disk in them you are changing the D-value, since the D-value is affected by the media and the media here is defined as whatever components are surrounding the spores. If you get an ampule that has spores and media, the D-value of this system includes spores, growth medium, and the glass of the ampule. Once again, if you open the ampule and use the spores suspended in medium as inoculum for a test, you have changed the D-value.

Questions from PMF Members

Have you seen D-value problems when using biological indicators exactly as given to you by a manufacturer?

New Product Launch

PML Microbiologicals is pleased to announce the introduction of sterile packaged sterility test media under the product name of *DuoTek System.* Included in this offering will be media in a sterile package impervious to VHP, for users of barrier systems. Literature and additional information may be obtained by calling your nearest PML facility at 800-435-5693 or 800-547-0659.

The Pharmaceutical Microbiology Forum would like to express it's appreciation and thanks to Applied Analytical Industries Inc. (AAI) for their continued support.

Purified Water System Survey

As part of the Pharmaceutical Microbiology Forum, we are collecting data via surveys. Please find a few minutes to fill out this questionnaire. Do not include your name or the name of your company. Send it to the Pharmaceutical Microbiology Forum, 1206 North 23rd St., Wilmington, NC 28405, C/O L. Valdes-Mora. The results will be published in the Spring PMF Newsletter.

Is your microbiology dept. responsible for:

1) Sampling the water system.

yes no

2) Water bioburden

yes no

3) Water chemistries

yes no

4) Is your incoming water from:

municipal water well water other

_____

_____

5) What components are in your purified water system?

mixed resin beds RO Membranes Still Other

_____

_____

_____

6) Are there any 0.1 u filters on your system?

yes no

7) Do you test for endotoxins?

yes no

8) What method do you use for endotoxin testing?

Gel clot Turbidimetric Chromogenic

9) What method do you use for bioburden testing?

Membrane filtration Pour plate

Other _____

_____

10) What medium do you use?
Standard plate count R2A medium

TSA medium Other _____

_____

_____

11) At what temperatures do you incubate the plates?

20-25C 30-35C 20-25C/30-35C

30-35C/20-25C Other

_____

_____

12) How many days do you incubate the plates?

_____ days

13) What is your testing interval?

Daily Weekly Monthly Other

_____

14) Is your purified water system validated?

yes no

15) How often do you sanitize your system?

Weekly Monthly Quarterly Other

_____

16) What sanitizing agent do you use?

hydrogen peroxide hot water formaldehyde

Other _____

17) What are your specifications for bioburden?

50/ml 100/ml Other

_____

Water Survey (continued)

18) Do you test for coliforms?

yes no

19) Have you ever isolated a coliform from your water?

yes no

20) In how many years of testing? _____

21) Did you determine if it came from a municipal water supply?

yes no

22) Do you test for total organic carbon?

yes no

23) If so how long have you been testing for TOC?

_____

24) What method or equipment do you use to test for TOC? _____

_____

_____

_____

25) What does your company produce?

pharmaceuticals cosmetics food

other _____

Classified Ads

Wanted: Microbiologist

Pharmaceutical company seeks experienced, responsible individual to perform the microbiological assay and sterility testing of oral and sterile dosage forms. Experience with semi-synthetic penicillins a plus. Salary commensurate with experience. Submit resume to :

President
Consolidated Pharmaceuticals
6110 Robinwood Road
Baltimore, MD 21225

Calendar of Events

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

1995

January 17-18, *PDA/ISPE Conference on Advanced Barrier Technology,* Atlanta GA

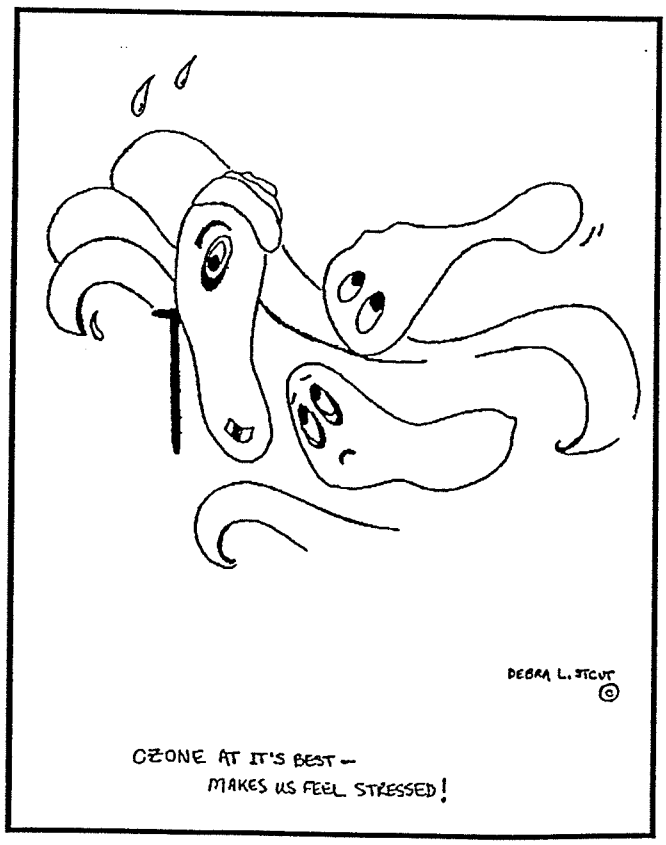
January 23-26, *PDA Lake Tahoe Courses,* Lake Tahoe, NV

March 12-16, *Third International Symposium on the Interface between Analytical Chemistry and Microbiology: Analytical Chemistry in Environmental Microbiology,* Knoxville, TN

March 13-17, *PDA Spring Courses, Meeting and Exhibits,* San Francisco, CA

April 26-28, *Applied Analytical Inc. (AAI), Validation in the Pharmaceutical Microbiology Laboratory: What's New in 1995?* , Wilmington, NC

MICRO TOONS



Pharmaceutical Microbiology Forum
Membership Application

Mission: PMF provides a forum for pharmaceutical microbiologists to exchange information on microbiological issues in the pharmaceutical industry, and interact with the USP and regulatory agencies.

Please Print

Name: _____

Title: _____

Company: _____

Address: _____

_____

_____

Phone: _____ Fax: _____

Optional:

Home Address: _____

_____

_____

Home Phone: _____

Membership Dues for one year are $15.00. Please send a check or money order to:

Pharmaceutical Microbiology Forum
C/O Elizabeth Darner
223 Sunnymead Road
Sommerville, NJ 08876

The PMF mailing list is private, and not for sale.

PMF Newsletter Comments
We value your input.
Please send questions or comments to:

Laura Valdes-Mora
AAI
1206 North 23rd Street
Wilmington, NC 28405

Newsletter is published by the Pharmaceutical Microbiology Forum, 1206 North 23rd Street, Wilmington, NC 28405. Telephone (910) 251-6786. Send all inquiries, letters, and comments to the above address, C/O L. Valdes-Mora.

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

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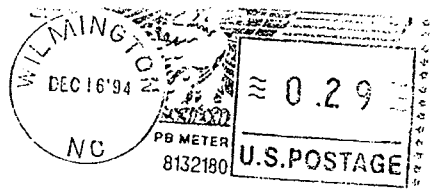

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Wilmington, NC 28405

Address Correction Requested

REMINDER! Have You Paid Your Dues? This is the last newsletter that will be sent to any member who has not yet paid their dues.