PMF NEWSLETTER

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Bacterial Endotoxin and Surface Sampling

This month's newsletter incorporates a range of topics in the discussion pieces. We have an excellent article on depyrogenation from Tim Sandle out of the UK which provides detailed guidance on the use of Endotoxin indicators to demonstrate the adequacy of a Depyrogenation cycle. This guidance is on a practical, common sense level that is sure to be of help.

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

As an aside, this will most likely be the last article on a bacterial Endotoxin topic, as the LAL User's Group Newsletter is about to launch. Anyone interested in hosting a User Group meeting at their facility (free training!) should contact Karen McCullough (karenzm@embarqmail.com) for more information.

The second article is a continuation from last month's discussion of microbial recover y studies. Last month we looked at microbial recovery in terms of method suitability studies. In these studies we are checking to see if two populations support growth of similar numbers of CFU. These could be preservative neutralization studies, media growth promotion, etc. This month we look at a different type of recovery study—recovery of organisms from a solid surface. This design is very different, and cannot be assumed to be capable of meeting a 70% acceptance criteria.

I hope you find this issue interesting and useful. The PMF is coming off the busiest year yet in terms of conferences, and has an even fuller calendar next year. Please let us know if you are interested in participating in either the newsletter or conference planning activities.

Scott Sutton scott.sutton@microbiol.org

Some Considerations on the Use of Endotoxin Indicators in Depyrogenation Studies

Tim Sandle, Bio Products Laboratory, UK

Introduction

Depyrogenation is an important factor in maintaining sterility assurance during the preparation of pharmaceutical products. There are several different means to achieve depyrogenation (including ultrafiltration; ion exchange chromatography and the use of acid-base hydrolysis). Arguably the most common depyrogenation devices are those which operate using dry-heat (such as a depyrogenation tunnel using unidirectional hot air and operating at 250°C for 30 minutes which is used to prepare primary packaging articles – product vials – for aseptic filling).

Depyrogenation can be defined as:

"The elimination of all pyrogenic substances, including bacterial endotoxin, and is generally achieved by removal or inactivation"
(PDA LAL User Group, 1989)

There are many different substances which can cause pyrogenic reactions (a pyrogen is simply substance that can cause a fever). However, the most common pyrogenic substances which pose a risk to pharmaceutical manufacturing are bacterial endotoxins (lipopolysaccharide (LPS) molecules found as part of the cell wall of Gram-negative bacteria).

Depyrogenation can refer to endotoxin inactivation or endotoxin removal. In the former the total destruction of the pyroburden is assumed (for example, using dry heat). For the latter the pyroburden is removed to a defined level (for example, rinsing). Both examples are accepted by the FDA as types of depyrogenation*.

Depyrogenation devices require qualification (as part of Performance Qualification) and routine verification. This is undertaken as a depyrogenation study. A depyrogenation study is a test of the physical capabilities of a device to depyrogenate an article or device. It is demonstrated by physical measurements (including temperature) and biological (using bacterial endotoxin applied to a suitable surface as an Endotoxin Indicator). This paper addresses the biological aspects, with a particular emphasis upon aspects of depyrogenation studies which are not defined in available guidelines, such as in USP <1211>.

Log reduction: three or four logs?

The biological aspects of depyrogenation are demonstrated using an Endotoxin Indicator. Depyrogenation, as defined by the USP and the FDA Guide to Sterile Drug Product produced by Aseptic Filling (September 2004), is demonstrated by a minimum of a three-log reduction of a minimum of a 1000 EU / article challenge (where an article is a glass vial / bottle or rubber closure).

Endotoxin, when dry heat inactivated, follows a linear log- reduction curve until reduction to three-logs. After this destruction continues to occur but does not necessarily follow linear regression (it is 'bi-phasic' reduction; Ludgwig and Avis, 1990; Hecker *et al*, 1994; Tsuji, 1978)[§].

Based on this phenomena, and additional information relating to the range of endotoxin recovery discussed below, many pharmaceutical manufacturers set a minimum criteria for a **four-log** reduction of at least 1000

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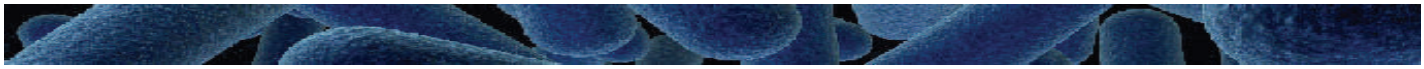
*DEPT. OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION (1985): 'ITG SUBJECT: BACTERIAL ENDOTOXINS/PYROGENS', Date: 3/20/85 Number: 40 at: http://www.fda.gov/ora/inspect_ref/itg/itg40.html (accessed 4th November 2005)

§ For example, the Ph. Eur. in 2.6.8 states two possible time-temperature combinations for depyrogenation: 60 minutes at 200°C or 30 minutes at 250°C. A quantity of endotoxin destroyed at 250°C for 60 minutes would not necessarily be totally destroyed at 200°C at 60 minutes, based on the non-linearity of the thermal destruction curve. Endotoxin destruction at low temperature is of the second-order. Ref: Ludwig, J. D. and Avis, K. E. (1990): 'Dry Heat Inactivation of Endotoxin on the surface of Glass', Journal of Parenteral Science and Technology, pp4-12, Vol. 44, No.1, January-February 1990; also, Hecker, D., Witthaeur, D. and Staerk, A. (1994): 'Validation of dry heat inactivation of bacterial endotoxins', PDA J Pharm Sci Technol, 48(4):197-204; also, Tsuji, K. et al (1978): 'Dry-Heat Destruction of Lipopolysaccharide, App. and Env. Microbial, 36:705-179



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EU per device. This is the recommendation of other practitioners like Hecker *et al* and Nakata (1993).

The risk of introducing a four-log reduction is that is exceeds the pharmacopoeia minimum requirement and that endotoxin may survive and a device may fail unnecessarily (although it over-comes to inherent difficulties relating to endotoxin recovery and destruction curve). However, a review conducted by the author of several depyrogenation studies in the UK indicates that the typical log reduction is >6 logs.

Endotoxin Indicators

The most important component in the biological assessment of depyrogenation studies are Endotoxin Indicators. Endotoxin Indicators are prepared using a preparation of Control Standard Endotoxin (CSE) from *Escherichia coli* O113:H10. The CSE used is similar to endotoxin used to perform routine the Bacterial Endotoxin Test (BET) using *Limulus* Amoebocyte Lysate (LAL) methodology in that:

a. It is a freeze-dried preparation of *Escherichia coli* lipopolysaccharide.
b. It is certifiable to an international reference preparation (Reference Standard Endotoxin).

The key difference is that the endotoxin is of a much higher potency (endotoxin concentration expressed as Endotoxin Units per ml [EU / ml]).

The preparation of CSE is sometimes referred to as 'high potency' or 'high concentration' endotoxin in order to distinguish it from endotoxin routinely used for LAL testing.

Preparation of endotoxin indicators

Although USP <1211> requires a minimum 1000 EU / device challenge per device, there is some debate within the industry regarding how an Endotoxin Indicator is prepared. As such, there are two possible ways to prepare an EI:

a. Adding a volume of endotoxin to the surface of the article to be challenged (where such a volume gives a concentration of ³1000 EU / device).

The endotoxin must be dried onto the surface of the article prior to challenging the depyrogenation device. This normally requires overnight preparation (in the experience of the author a 0.1 ml volume of endotoxin will take > 8 hours to dry onto the surface of glass or rubber when held under a UDAF at ambient temperature).

Once dried, the article(s) are placed into the depyrogenation device.

b. By placing a vial of high potency endotoxin (unreconstituted) directly into the depyrogenation device. Following the completion of the depyrogenation cycle, the vial(s) are removed, reconstituted and test to quantify the remaining level of endotoxin.

Of the two approaches, the **first approach** the recommended one and, from experience, the most likely to satisfy regulatory agencies. This is because it is considered the greater challenge as the endotoxin is applied directly to the surface of the device to be depyrogenated.

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Validation of Microbial Recovery from Surfaces

Scott Sutton, Ph.D.

Introduction

Last month we looked at recovery studies designed to show differences between two putatively identical populations. These could be an old batch of media versus a new batch of media, an inoculum into a neutralized disinfectant compared to its control, or other comparisons that we expect to be the same. We have established a 70% recovery (test sample to control sample) as our criteria for these studies, unless mandated by compendial directive.

A second type of recovery study that is commonly done is to evaluate the recovery of microorganisms from a solid surface. This might be done to aid in the development of a minimal sterilization cycle for a medical device, it might be done as part of a disinfectant study, to qualify a method of taking environmental monitoring samples, or for a number of other reasons.

Since 70% recovery is expected for microbial validation studies, we should insist on this acceptance criterion for this study as well – right? Actually, we probably should consider that the 70% criteria came from a completely different experimental design, with completely different conditions. Despite these differences, there is a belief that "everyone knows it is in the USP" that 70% is the acceptance criteria for recovery studies of surface sampling methods. This turns out not to be the case. One must exercise caution in uncritical acceptance of "what everybody knows". In addition, it is always useful to bear in mind that the compendia (USP, Pharm Eur, JP and even the BP) are written by mortals and may not actually be correct for your application. However, in this case, the USP at least is silent in regards to reasonable acceptance criteria for a surface recovery study.

So, if I am saying 70% is not a reasonable acceptance criterion for surface recovery studies, what is? Before answering this question, let's take a step back and try to figure out how to design a study to show microbial sampling frequency from a surface.

Study Designs

Deposition of Organisms

The first challenge in developing a test to measure bioburden sampling recovery from a surface is to get some bioburden on a surface to sample. The first thought is to do this like we do most tests, grow some cells up in culture to use as an inoculum. We spread the cells on the solid surface, allow them to dry, and then sample them. A problem with this approach is that vegetative cells are susceptible to "death by dessication" to varying degrees (Potts 1994). In other words, your inoculum is dying off at an unknown rate during the time you are conducting the experiment. This situation does not seem to be well-designed to yield accurate results. One way of approaching this problem I have heard about is to sample before the inoculum completely dries on the surface. This cer-

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tainly helps with the dessication problem, but I have never been able to figure how sampling a puddle is relevant to the question at hand.

Perhaps the problem is the organism used. Bacterial spores are easy to come by and are resistant to dessication. Several procedures use spores of organisms from the *Bacillus* genus for these studies. This is a valid approach, but we must remember that this is valid only as a lab assay – it does not reflect contamination as it is likely to involve a more firm attachment of the cells to the surface. A firm attachment of cells is easy to envision in terms of a nascent biofilm of growing cells on a moist surface. This concern is probably less of less importance in a clean room where we might reasonably assume that most contamination is the result of a transient deposition of organisms from the clean room operators, rather than an established biofilm firmly attached to the surface. So perhaps we might be able to defend deposition of spores as a reasonable model for clean room surface contamination.

A different approach might be to take materials of construction from the facility and allow them to be contaminated naturally. This, however, leads to questions about how to calculate recovery if we do not know the starting concentration.

For the moment at least, let's assume we will deposit bacterial spores of some type on the surface to be tested and allow the inoculum to dry thoroughly.

Sampling for Enumeration

Contact plates are very useful, but they do have some drawbacks. One of them is we don't know how effective they are on all surfaces (that's what we are trying to find out). Another is that they are of finite size. This size is usually smaller surface area than that of a standard petri dish. The first problem to think about then is how many CFU can actually be counted on one of those things. The 25 – 250 rule was developed for *E. coli* on standard plates (reviewed in *PMF Newsletter* of September, 2006 at http://www.microbiologyforum.org/PMFNews/PMFNews.12.10.0610.pdf). With a smaller surface area the contact plate cannot support the same numbers – but what numbers are allowed in your SOP?

A second problem here is that you will frequently want to use this technique to qualify disinfectants, sanitizers or sporicides for hard surface use in your facility. Very difficult to show a 3-log reduction if you cannot dilute the solid surface, nor can you count 1,000 CFU on a plate.

This limitation must be kept in mind while we look at available designs.

Available Designs

Direct inoculation and sampling

Having pointed out the shortcomings of direct inoculation, I am now going to recommend it as a viable design for surface recovery studies. The fact that it

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Technician qualification (recovery experiments)

Prior to performing a depyrogenation study, it is recommended that each technician undertaking the test performs an endotoxin recovery study. This involves preparing test bottles using the same methodology as the positive controls (see below).

The purpose of these experiments is to:

a. Qualify the technician;
b. To confirm that the lysate and CSE are satisfactory prior to performing the main study;
c. Establish the recovery range.

Although the recovery methodology is designed to recover the highest level of endotoxin possible, there is no pre-set criteria in any guideline for the level of recovery expected other than to provide a challenge of >1000 EU per device. The recovery experiment is designed to demonstrate that a *consistent level* can be recovered. The methodology employed typically includes ultrasonication and vortex mixing.

When conducting recovery tests the percentage recovery for each test should be calculated from:

Step 1

Theoretical endotoxin challenge (A):

$$\left(\frac{Potency\ of\ high\ concentration\ endotoxin\ in\ EU}{Volume\,(mL)\,used\ to\ reconstitute}\right) = A1$$

$$A1\ x\ (Inoculation\ volume\ \ per\ device) = A$$

Step 2*

Recovered endotoxin (percentage recovery) (B):

$$\left(\frac{Actual\ endotoxin\ measured}{Theoretical\ endotoxin\ challenge\ (A)}\right) x\ 100 = B$$

The percentage recovery from the three experiments should be analysed to determine which has given the lowest recovery[§] . This establishes the 'worst case' scenario for the actual test runs. At times the figure for the percentage recovery may be higher than the theoretical challenge if residual endotoxin is present in the test vials.

Placement and number of EIs

Once prepared, EIs are placed in defined locations in a depyrogenation device. These locations are typically determined by a number of thermometric runs to determine cold spots. The number of EIs used is based on the size of the depyrogenation device and this can be a subject of much debate (with the capacity of the testing laboratory often a limiting step). Typically, 5 – 10 EIs are sufficient to assess the depyrogenation capabilities of most devices undergoing testing.

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* The estimate of the actual endotoxin recovered (measured) is based on the mean of test replicates multiplied by the dilution factor.

§ This is the mean of the test vials. Each recovery test run normally involves two replicates.

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It is important to maintain aseptic technique when placing and removing the EIs from the dehydrogenation device.

Positive and negative controls

When conducting depyrogenation studies it is important to include positive controls in the test set. Whilst the amount of endotoxin added to an EI is ³1000 EU / article. This is only a theoretical level of endotoxin based on the accompanying Certificate of Analysis from the endotoxin supplier. In practice, the actual level of endotoxin must be confirmed by determining the level of endotoxin that can be recovered from the article challenged.

This is performed using positive controls. These controls should be prepared at the same time as the EIs. They are not placed inside the depyrogenation device and are tested at the same time as the EIs (post-depyrogenation).

There is no guideline as to the set amount (percentage) that must be recovered from the positive controls compared to the theoretical challenge. What is important is that the challenge can be demonstrated to be ³1000 EU / article. The reason that there are no set criteria is a reflection of the variability associated with endotoxin recovery. As way of an indication a study by Plant gave a range of between 20 and 70% as the typical recovery range.

There are different reasons for such variability outside of the limitations of the LAL assay. These can relate to the different potencies of endotoxin and thermal death kinetics (non-linear thermal reduction) of environmental endotoxin from different Gram-negative bacterial lipopolysaccharide. Because of the variables of non-linear thermal reduction; environmental endotoxin and different recovery rate support is lent for a **four-log** reduction of endotoxin.

When testing of positive controls the same methodology applied to the EIs should be used, as the approach is designed to recover any remaining endotoxin. Positive controls require diluting to an estimated level that will fall within the range of endotoxin concentrations on the standard series.

Positive controls, therefore, serve three purposes:

a. To confirm the actual (recoverable) endotoxin challenge to the articles.
b. To demonstrate that the methodology used to test the post-depyrogenation EIs is capable of recovering any remaining endotoxin.
c. To confirm that any endotoxin present on the EIs can be recovered following the period of time after their removal from the depyrogenation device (that is the expiry time has not been exceeded).

The theoretical quantity of endotoxin and the recovered level of endotoxin (from the positive controls) normally differ, with the recovered level of endotoxin typically being a lower value. This is due to endotoxin being adsorbed onto the surface of the article and not all of this can be recovered. However, on occasions the level of recovered endotoxin may appear

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as higher than the theoretical level. This is due to the acceptable range of variation with the LAL assay (at 50 – 200%, as detailed in *Ph. Eur*. 2.6.14).

It is also recommended that negative controls are used with each study. Negative controls used in the test are articles, which have not been challenged with endotoxin. These are tested to demonstrate if the articles contained any residual level of endotoxin or if any exogenous endotoxin has been deposited into the vials or bottles being tested.

Endotoxin Standard Series / Methodology

After completing a depyrogenation study the test bottles must be examined against the LAL test. The laboratory may wish to give consideration to suitable expiry times for the articles post-depyrogenation.

With respect to test methodology, although gelation methods can be used the examination can be conducted far more easily using either the kinetic turbidimetric or chromogenic methods. In preparing the standard series for the test it is important that the endotoxin standard series used to assess the EIs is prepared from the vial of CSE used to challenge the EIs prior to starting the depyrogenation study. When preparing the endotoxin series it is important that the standard series covers a minimum of three-logs and that it is capable of demonstrating if a three-log reduction of the EIs has occurred.

The EIs (post-depyrogenation), positive controls and negative controls should be tested as part of the same LAL test. The recovery method involves the addition of pyrogen-free water (LAL Reagent Water) to the article, followed by a combination of rinsing, vortex mixing and ultrasonication.

The solutions from the articles are then tested against the standard endotoxin series prepared from the endotoxin vial used to prepare the EIs. Each laboratory will have its own range for the standard series, for example, a standard endotoxin series covering the range 5.0 to 0.005 EU / ml.

Once the study is complete the log reduction must be calculated.

Log reduction calculation

To calculate the log reduction (E):

1. Calculate the mean level of endotoxin recovered from the positive control bottles
2. Multiply this value by the minimum recovery from the recovery experiments (B1) to yield the intermediate result "C"
3. Divide the value "C" by the mean of the post-depyrogenation endotoxin concentration from the test vials to yield the intermediate value "D"
4. E = the $\log_{10}$ value of D

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probably has little relation to the actual attachment of bacteria to a surface is only of passing concern. First of all, as we mentioned before, people are going to be the primary source of contamination in a well-maintained cleanroom. People shed skin at a prodigious rate (on this scale) with an entire layer replaced on average in less than 24 hours (Jansen et al 1974). These particles can be measured and a normal individual can shed enormous numbers of skin fragments. Many of these will contain viable bacteria (Ljungqvist and Reinmuller 2000) and these will, in all likelihood, be the primary material recovered from surface monitoring (as well as both passive and active viable air sampling).

This assumption (that clean room contamination will be primarily from the operators shedding skin flakes onto surfaces) allows us to justify the use of a controlled inoculum to be placed in a precise area of the coupon. Several studies have used this approach to determine recovery efficacies (see below). However, lets look at a couple of other designs before moving on.

Linear Regression

A different approach is to use a linear regression model (Whyte 1989). The inoculation design provided us with a starting inoculum from which we can estimate recovery frequent by taking the recovery from the first sampling and dividing the CFU recovered by the total. In this method, a surface is sampled repeatedly and the number of CFU recovered is graphed against the sample number on a log-normal scale. The slope of the resulting line is $log_{10}(1-Sampling\ Efficacy)$. This method has an enormous advantage in not requiring knowledge of the number of organisms originally on the surface. This consideration is particularly important if the surface recovery study is being used to prepare for a sterilization cycle of a medical device where you usually have little idea of the native bioburden.

Sampling to Extinction

A less mathematically-oriented approach that provides much the same advantages is recommended by ISO (ISO 11737-1). In this approach the same area is sampled repeatedly (as many as 10 times to ensure that no CFU are recovered in the later sampling events) and each sampling event is incubated for recovery. The CFU form the initial sampling event is then divided by the total of the recovered microorganisms for the recovery frequency. Like the linear regression method this does not require knowledge of the initial contamination level, but has a huge advantage in only requiring basic math skills.

Acceptance Criteria

So, after all this, what are reasonable acceptance criteria? I don't know. There are too many variables and the point of the study is to determine recovery frequency.

Favero (1968) describes several different methods:

- Swabs
- Rinses
- Pads
- Contact Plate
- Roller Plates (agar sausage)
- Flexible Film
- Adhesive tape

These are all going to have different recovery efficiencies. In addition, even using the same method, different challenge organisms are likely to provide different estimates of efficacy (Douglas 1968). Each must be evaluated on its own merits. As an aside, recovery efficacies in the literature seem to range from 10 – 70% depending on the organism, surface, and sampling method.

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Upcoming Events

February - Open Conference on Compendial Issues (Scott Sutton with representatives of the USP subcommittee).
Baltimore, MD

March - Environmental Monitoring (Susan Schniepp, moderating)

April - Validation Issues in Microbiology
Philadelphia, PA

May - Microbiology Investigations (Frank Settineri, moderating)

June - GMP for the Microbiology Lab (Scott Sutton, moderating)
Dallas/Ft. Worth, TX

September - Cosmetic Microbiology
Newark, NJ

October - 2008 PMF Fall Forum (Scott Sutton, moderating)
Rochester, NY

November - Bacterial Endotoxin Summit (Karen McCullough, moderating)
New Brunswick, NJ

Offering In-House Courses on Microbiology/Aseptic Processing:

- The Microbiology Network/High Peaks Associates http://www.highpeaks.us/in-house.htm
  Experienced in custom-designed courses for the lab or the manufacturing facility, including
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  - Auditing the Microbiology Function
  - Investigating Microbiological Data Deviations
- USP
  Contact Steven Paul (stp@usp.org) for information on the course "Fundamentals of Microbiological Testing"

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.

Discussion List Update

PMFList:
Number of Subscribers: 2682
Number of Countries: 63
Number of Messages Last Month: 468

PSDGList (Pharma Stability Discussion Group):
Number of Subscribers: 1034
Number of Countries: 23

Membership is FREE. To **join the PMFList**, visit http://microbiol.org/pmflist.htm and register.

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. Archives of the lists are available at:

- http://lists.microbiol.org/archives/PMFLIST.html
- http://lists.microbiol.org/archives/PSDGLIST.html

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It is also important to ensure that the LAL test is valid and meets the compendial criteria for the standard curve (r = >0.980).

Summary

This article has briefly considered some of the important factors relating to the use of Endotoxin Indicators used to assess the effectiveness of depyrogenation devices. If one important message can be drawn out from the paper it is the importance of technician qualification and in practising the recovery method. Time spent with method development will pay dividends when the time to conduct the study arrives.

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None of this helps if you are attempting to use a surface recovery design to "validate" a surface monitoring procedure for a facility where you are concerned about residual sanitizer. Clearly this is a concern. I would urge you to consider, however, that given the vagaries of the sampling technique, this approach is not the shortest path to your goal (or even the one most likely to be successful). I would recommend demonstration that a volume of the sanitizer placed onto the agar surface and allowed to "soak-in" is adequately neutralized and that the medium is capable of supporting growth of a later inoculum by the means we discussed last month. This is not an approach you will find in any regulatory document, but it will allow for a meaningful and interpretable study of the growth medium's neutralizing capability.

Conclusion

Do not allow yourself to be committed to acceptance criteria that are unreasonable and will doom the study to excessive deviations. A poor choice of unattainable acceptance criteria makes what should be a relatively straightforward study very difficult to close-out. The surface recovery study can yield important information of great use to the environmental monitoring program, evaluation of surface disinfectants/sporicides, determination of native bioburden for medical devices and other applications. However, it is critical to ensure that the study is asking the correct question, and that the design is appropriate to answer the question being asked. Above all, make sure that established acceptance criteria for the study are well thought-out and achievable.

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2007 PMF Bacterial Endotoxin Summit

http://www.highpeaks.us/2007/BES/index.htm
November 8-9, 2007
New Brunswick, NJ

The FDA LAL Guideline is 20 years old! While the USP is clearly the most current and technically correct testing document, there are many **GMP topics** in the Guideline and beyond the Guideline that still need discussion.

Last year, the BES explored the basics of the LAL test. The focus of this year's meeting is to provide a forum for the sharing of questions, answers and information **on GMP aspects of the Bacterial Endotoxin Test** (BET).

This two-day interactive program includes case studies, problem solving and round table discussions. In addition, we'll have a mini exhibition, where vendors of BET testing reagents and equipment will all have their products on hand for viewing by participants.

Topics

- The Big Moving Parts of Depyrogenation - Ron Berzofsky, Ph.D.
- How Much Testing is Enough? Determining Critical Control Points for Process Control - Karen McCullough
- Data Analysis: Setting Action and Alert Levels - Alan Baines
- Water System Failure - What's the Risk? - Mick Dawson, Ph.D.
- OOS and Investigation - Does the 2006 FDA OOS Guidance Apply to the BET? - John Dubczak