PMF NEWSLETTER

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Letter from the Editor

Welcome to the September issue of the 2012 *PMF Newsletter*.

This issue presents a unique perspective offered by Tony Luttrell on QC Microbiology laboratories . Mr. Luttrell is a highly experienced and well-known Quality Assurance consultant, and offers his insight into many issues he has encountered in his career. His thoughtful advice is a "must read" for QC Microbiologists interested in the views of a QA expert.

Don't miss!

PMF Conference on Environmental Monitoring

December 15-16, 2012

http://www.cvent.com/d/vcq207

Dr. Tim Sandle returns with an article summarizing the proposed revision to the European Pharmacopeia chapter on Biological Indicators. The revision is effectively a complete rewrite of the chapter. Dr. Sandle expertly summarizes the revision points that will impact the way in which biological indicators are used by companies which are required to conform to the European Pharmacopoeia.

We are always interested in your input and concerns. Please let us know if there are articles or topics you would like to see addressed in this newsletter.

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Training on the Right Things in the Microbiology Laboratory

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Over the years, being in and out of many microbiology laboratories performing audits, assessments and remediation work, I continue to be struck at how some of these places are like the "land that time forgot." While I find many well-meaning laboratory analysts and supervisors, some of these labs have just plain been neglected. This causes all kinds of dissonance when a knowledgeable regulatory investigator enters the scene with a multi-page citation. Don't kid yourself… this happens more than Management wants to admit.

What I hope to offer with this brief article is a list of basic things to watch out for, to train on and things that can be easily attended to, with immaterial incremental cost to the Firm.

1. Cleanliness, the First Principle

You cannot expect quality work to be conducted in a cluttered and dirty laboratory. You must declutter and clean and wipe constantly. It always amazes me when I wander into a laboratory function and I see cardboard, clutter, paper and equipment strewn around the work space. In one laboratory I walked into, I found soda-pop cans! This is especially true for a work area where live organisms are being handled, pure cultures are being kept and where environmental media is being incubated. Time after time, I have observed that investigations into media or culture contamination trace the root cause back to cardboard boxes or sleeves that may have set out on the tarmac at Dallas Ft. Worth Airport prior to arrival to your loading dock and then being immediately shipped to your laboratory, where those *Aspergillus* spores keep on germinating on that tasty and damp paper. Next thing you know, it's on your lab coats, on your analyst's hands and on your benches. When things are cluttered and crowded you just simply cannot clean properly (even <u>if</u> you are convinced that the spritzer spray of 70% isopropyl alcohol is really doing the job!).

Big ideas for Training and Control

A. Do not allow cardboard and other paper into the immediate work area. Require that materials be deboxed and stored only in plastic (which should also be wiped with disinfectants when entering into areas where "clean work" is being done). Also keep those favorite college textbooks about clinical microbiology and papers and files out of the immediate work area. If your laboratory has paper procedures issued from your Document Control function, sleeve them in plastic. Use the rule of thumb that if you don't use it regularly, store it away or get rid of it completely. Strive to keep a simple and functional work space.

B. Clean and disinfect every day, or after every operation when organisms are handled. Disinfect counters, refrigerators, incubator interiors, cold rooms (ever see mold growing in a home refrigerator even at 2 - 8°C?) and most importantly, unidirectional flow hoods and biological safety cabinets. Use a disinfectant that is appropriate for the organisms being encountered. Microbiology laboratories should employ compatible and rotating sanitizers and disinfectants, in the same manner as those employed in manufacturing areas. Sporocides should be a

(Continued on page 3)

(Continued from page 2)

routine part of the disinfectant rotation. Mop the floors every day with an approved agent. Put explicit instructions for proper overlapping strokes and use of clean applicator systems (wipes and sponge mops) in your local procedures. Most of all, do not make assumptions that your staff microbiologists are doing the cleaning properly even if they have read the SOP. No one likes to do cleaning except a rare few individuals. Cleaning should be demonstrated and checked, then documented. Assume nothing in this area, check everything. It will make that upcoming investigation a lot easier when you can prove that your lab is in control.

C. Put on clean lab coats every day and gown properly. It just never ceases to amaze me that microbiologists run around the laboratory with a soiled lab coat, moving from the "hot areas" to "clean work." Don't let those lab coats out of the microbiology area. Using common sense helps, like shoe covers and hair nets for work, whenever practicable. Use every common sense help to keep the contributory bioburden down. Never underestimate the effect of clean clothing (including clean shoes) and a daily shower (hygiene)!

2. Qualifying Analysts on the Obvious Things

Well run laboratories have methods for demonstrating that the "obvious" activities in a laboratory are trained on and documented in a skill based assessment ("training-ease" for demonstrating that you can actually perform a Gram stain). Skill-based programs should be developed for every activity that requires a laboratory technique and an interpretation. These include basic things like plate streaking, slide prep, even looking under a microscope. Even though you may have hired smart microbiologists, it is like anything else, if you don't do it every day and practice, you cannot master it. I have been surprised more than once to discover that people actually cannot do a Gram stain correctly. On that point, the best run labs prep and run lots of unknowns, on a regular basis. Keep representative slides in a library for references. Prepare and submit cultures regularly for ID and Gram stains (this is analogous to running system suitability on analytical equipment). Unknowns should be run for activities such as Gram stains, microbial ID's, identification by morphology keys, such as for molds, etc.

3. Qualifying and Understanding Compendial Methods

Thematically, I continue to find that more than a few analysts and microbiologists are not familiar

(Continued on page 6)

European Pharmacopeia Proposes Revision to Biological Indicators Chapter

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The European Pharmacopeia (EP) is proposing to revise the chapter within the pharmacopeia relating to 'biological indicators' (chapter 5.1.2, chapter entitled "Biological indicators in the preparation of sterile products"). A draft for public comment has been published in the most recent edition of *Pharmeuropa Bio and Scientific Notes* (Edition: 24.1 January 2012). Pharmeuropa, like the EP, is published by European Directorate for the Quality of Medicines and HealthCare (EDQM) and seeks to publish "public inquiries on draft European texts or on matters of general policy".

The revision has been proposed as part of the cycle of updating pharmacopoeial chapters. Upon review the chapter was considered to be out-of-date, particularly in relation to the technical content. The draft text presented in Pharmeuropa is effectively a complete rewrite of the original text.

Interestingly, an ahead of proposed developments with the USP chapters pertaining to sterilization*, the scope of the chapter is extended to include indicators for depyrogenation studies. These are commonly been described as 'endotoxin indicators' in many texts; here, the EP is categorizing 'endotoxin indicators' as 'biological indicators'.

Within the draft a new definition of biological indicators has been devised. A biological indicator is defined, somewhat succinctly, as: "test systems containing viable microorganisms providing a defined challenge to a specified sterilisation process".

Three different types of biological indicator are described:

- Inoculated carriers (the classic paper spore strip or a metal carrier).
- Self-contained biological indicators (such as ampoule containing a population of the test microorganism in an appropriate nutrient medium).
- Custom-made biological indicators (although only where no commercially produced biological indicator can be manufactured for the particular application).

With custom-made biological indicators, the EP advises the user to consider:

- The drying time.
- The inoculation method.
- The representativeness of the surface being inoculated.
- Wrapping procedure and material.
- Measures to be taken to ensure that the inoculated spores are not removed prior to use.
- The temperature profile of the inoculated material relative to other items subjected to the sterilization cycle.
- Spore recovery post-sterilization.
- Effectiveness of the culture media used for the spore recovery.

A further change is that biological indicators are no longer recommended for use in radiation sterilization

(Continued on page 5)

**USP chapter <1211> "Sterilization and Sterility Assurance of Compendial Articles" is currently under review, along with a new and more detailed chapter looking at sterilization methods: <1229> "General Concepts for Sterilization.*

(Continued from page 4)

cycles.

For microbial recovery, no culture media is recommended with a reference made only to a "suitable" medium.

The responsibilities for manufacturers of biological indicators and the users of biological indicators are outlined. However, the long-running debate as to whether biological indicators need to be independently verified is left unresolved. The text states that the user may either audit the manufacturer or have each lot of biological indicators independently characterized and that the choice between these two options must be justified.

Manufacturers are instructed to prepare biological indicators with a stated purity, population and D-value, along with the methods to determine resistance. The assumption is that this applies to all biological indicators for all applications, including gaseous sterilization.

With steam sterilization the D-values for biological indicators is extended from 1.5 to 3.0 minutes, to 1.5 to 4.5 minutes. This may be in recognition to the 'creeping resistance' in terms of biological indicator resistance. In relation to dry hear sterilization, the EP proposal is to use biological indicators which have a D160°C value of about 2 min to 5 min.

A significant change with the pass/fail criteria for a sterilization process is introduced. In the current chapter a successful sterilisation process should leave no revivable spores. However, in the proposed revision it states that the process shall deliver such a lethality to the selected BI that the survivor rate is between 1 per 10 BI units and 1 per 10,000 BI units.

Another change, which is not gone into in any detail, is the option for so-termed "highly effective sterilisation processes", not to perform biological indicator validation at all, as captured by the text "decision not to perform biological validation must be justified". This will be a tricky area in terms of regulatory compliance.

In terms of the microorganism used to prepare the biological indicator for the validation of steam sterilization, the chapter now suggests that the microorganism typically used, *Geobacillus stearothermophilus*, may not be suitable for sterilization processes delivering an F_0 between 8 and 15 and a different test microorganism should be used. For dry heat sterilization, *Bacillus atrophaeus* is recommended for temperatures above 160°C. However, no recommendation is made for the microorganism to be used at lower temperatures.

The draft discusses some specific sterilization processes:

(Continued on page 7)

(Continued from page 3)

with the basic USP/ EP methods and the requirements detailed in the compendia. For example, there are very specific requirements for inocula, organism type and recovery. Review of the relevant USP or EP section should be part of the basic analyst qualification curricula and much of what goes on in a routine pharmaceutical or biopharmaceutical laboratory is driven by guidance in the compendia. Secondly, all compendial methods need to be exercised and the results documented so that the local laboratory can demonstrate that they are able to execute the method. The process to qualify these methods should be formal and formally documented, such as in a protocol. Similar to basic laboratory operations above, these methods need formal skill-based training and the requisite documentation.

4. Use and Qualification of Media Suppliers

Most microbiology laboratories are purchasing media "ready to use" for the obvious ease of use and the hassle and controls needed to make it yourself. These suppliers need to be qualified formally and analysts should be trained that only media from qualified suppliers are allowed to be used. In addition to a formal Quality Assurance assessment of your suppliers, at least for sterility testing media and environmental monitoring supplies, good practice is to validate the vendor expiration dating. This is usually done by keeping materials past expiration date (e.g., two weeks to a month) under the prescribed storage conditions, and then conducting growth promotion testing with the designated organisms.

5. The Use of Positive and Negative Controls

Analysts need to be trained to always run positive and negative controls for most, if not all, microbiological analyses. Additionally, training should be conducted so that microbiologists know what to do when controls do not work. What is the impact to the test or by extension, to the series of tests that have been run, as well as the potential impact to the batch or lot of material being evaluated?

Just doing some of these simple things and training staff microbiologists will dramatically increase control in the laboratory and allow the laboratory to run under a greater state of control. Finally, these hints cost very little incrementally and will allow you to convince others, such as regulatory inspectors, that your lab is in compliance cGMPs!

About the Author:

Tony Luttrell is CEO and Co-Founder of Luttrell Consulting Group (www.luttrellgroup.net). He has over thirty years experience in the pharmaceutical, biopharmaceutical, and medical device industries. He specializes in operations management and quality systems for active pharmaceutical ingredients, finished pharmaceuticals, medical devices, and biological products. He has extensive experience in all aspects of manufacturing, validation, Quality Control, computer systems compliance, plant microbiology, and Quality Assurance in a GXP environment.

Tony has held numerous positions of increasing responsibility in industry from the laboratory bench to executive management, including most recently, Vice President of Quality for MedImmune, Inc. Previously, he held executive positions including Vice President of Quality and Vice President of Operations. He has also been Director of Quality Assurance for several companies. He previously worked for Solvay Animal Health, Kendall McGaw Pharmaceuticals, Baxter Healthcare and the Warner-Lambert Company.

(Continued from page 5)

- For the validation of container-closures (stoppers) the draft chapter makes a recommendation that spore suspensions be used, whereby the stopper is inoculated directly. The chapter states that commercially prepared suspensions may be used or that the user may use environmental isolates. With the use of environmental isolates no guidance is given about a suitable microorganism type or how a spore suspension is to be prepared (or verified).

- For the assessment of dry-heat sterilisation the text proposes that validation be performed with the sterilization devices running 10°C lower than the process temperature in order to assess 'worst case'. BIs for gas sterilisation.

- With gas sterilization, the chapter indicates that due to the range of different types of gas sterilization (including ethylene oxide, formaldehyde and low-temperature steam, hydrogen peroxide, and peracetic acid) and in the absence of any 'reference cycle', that no criteria will be defined. Instead, each user must define the cycle and select the appropriate biological indicator.

- A test for the microbiological evaluation of membrane filtration processes is introduced. This is the first substantive mention within the EP about sterilizing grade filter validation, although little detail is provided other than the statement "Membrane filtration is applied to reduce the bioburden of products prior to a heat sterilisation, or as a final step in an aseptic production process. A suspension of vegetative cells is typically used for the microbiological validation of membrane filters." The recommended microorganism, *Brevundimonas diminuta*, matches most literature references, with a challenge of at least 10^7 CFU per square centimetre of filter surface for filters with a pore size of 0.22-0.2 µm. For filters with a pore size of 0.1 µm the suggested microorganism is *Acholeplasma laidlawii* (ATCC 23206).

With the endotoxin challenges there has been a longstanding debate about whether the indicators should be made 'in-house' whereby endotoxin is applied to the types of vials being validated or whether commercial preparations can be used. The EP appears that it will allow either to be used.

Access to the EDQM site, and for Pharmeuropa, requires a subscription which is free of charge but must be completed in order to make **c**omments.

The new text, if adopted, will change the emphases in the way in which biological indicators are used in territories which are required to conform to the European Pharmacopoeia. With the USP sterilization chapters in development it is unfortunate that the opportunity for harmonization has been overlooked.

About the Author:

Dr. Tim Sandle is the Head of Microbiology at Bio Products Laboratory Limited. In addition, Dr. Sandle is an honorary consultant with the School of Pharmacy and Pharmaceutical Sciences, University of Manchester and is a tutor for the university's pharmaceutical microbiology MS course. Dr. Sandle serves on several committees relating to pharmaceutical microbiology and cleanroom contamination control including BSI cleanroom standards and the UK Pharmaceutical Microbiology Interest Group. Dr. Sandle has written over one hundred papers, book chapters and technical articles and he also runs an online microbiology blog (www.pharmig.blogspot.com).

Upcoming Events

PMF Conference on Environmental Monitoring - Major Challenges in Environmental Monitoring: A Systems Approach
December 3-4, 2012 - Las Vegas, NV
http://www.cvent.com/d/vcq207

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.

The *Pharmacopeial Forum* is now available for free online at http://www.usp.org/USPNF/pf/

Discussion List Update

PMFList:
Number of Subscribers: 4390
Number of Countries: 64
Number of Messages Last Month: 168

PSDGList (Pharma Stability Discussion Group):
Number of Subscribers: 1593
Number of Countries: 35

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